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(57) Abstract

A pharmaceutical composition based on blood drawn from a human suffering from leukemia which comprises a fraction obtained by removing in a known manner the corpuscular elements of blood drawn from a human suffering from leukemia, and optionally a pharmacologically acceptable adjuvant. In vivo and in vitro experiments have demonstrated that various components of leukemic blood have tumor inhibiting effect well above the threshold of significance.

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PHARMACEUTICAL COMPOSITION BASED ON BLOOD DRAWN FROM LEUKEMIA PATIENTS

The present invention relates to an improved composition based on blood drawn from patients suffering from leukemia and to its use in the human therapy for stimulating the immune system, mainly for the therapeutic and after-treatment of tumorous diseases, for the treatment of tumorous diseases in the starting fraction and for the prevention of the formation of new tumors, furthermore for the stimulation of a prostrated immune system.

It is well known that in addition to the traditional surgical interventions and radiological and/or chemotherapeutical treatments therapeutic methods based on the stimulation of the immune system are coming increasingly into prominence. Without an aim at completeness reference is made for example to treatments with interferon, to therapeutic methods based on the use of tumor necrosis factor, to treatments with antitumorous antibodies, or to immunostimulating methods carried out with pathogens like chicken plague. It is also a fact that the known methods based on the stimulation of the immune system did not produce resounding practical results. In view of the fact that the number of tumorous diseases and of diseases connected with the prostrated immune system is necessarily increasing due to the fortunate increase of average life expectancy, there is a continuous need for pharmaceutical compositions and for methods useful for the treatment of diseases of the above-mentioned types on behalf of practitioners in the human therapy .

It was disclosed in Zentralblatt für Gynecology, 19, 634 to 639 (1971) that in combination with a radiological treatment, immediately following this treatment, blood drawn from patients suffering from leukemia, particularly both from lymphoid and myeloid leukemia, can be used intramuscularly for the treatment of collum carcinoma (ovarian cancer, stomach cancer and cervical cancer) of 3rd stage provided that said blood is subjected to an incubation of differing time length and temperature, depending on the exact timing of the subsequent therapeutic treatment, and to a usual stabilizing treatment against clotting. Said publication was drafted on

a very low case number. On the basis of these examined cases one could, however, establish that the blood therapy was effective solely together with radiation therapy, with a beginning of this therapy immediately after the termination of the radiation therapy because if said blood therapy was started four months after the termination of the radiation therapy, the patient passed away.

According to the above publication the incubation of the blood, drawn from patients suffering leukemia and stabilized with sodium citrate in usual manner, was carried out with differing time lengths and temperatures, depending on the exact timing of its therapeutic use (in one case first at room temperature for 24 hours, then at 0 - 2 °C for 4 days, and in another case first at room temperature for 24 hours, then in a refrigerator for 3 days). It is quite understandable that in the every day's clinical practice such an incubation method is disliked. The above publication contains a hint to the fact that one has to secure the identity of the blood group in case of the blood used for treatment. This precondition renders the applicability of blood, being obtainable from patients suffering from leukemia with great difficulty, even more difficult. The presence of shaped components in the composition can also be considered as detrimental from the point of view of preservation.

Due to the above shortages of the solution according to said publication, primarily in view of the non-convincing character of the test data, the practitioners in the present therapeutic field did not accept this method, no further experiments were carried out in this respect and the usability of blood drawn from patients suffering from leukemia was forgotten.

Thus, the present invention relates to a pharmaceutical composition based on blood drawn from a human suffering from leukemia which comprises a fraction obtained by removing in a known manner the corpuscular elements of blood drawn from a human suffering from leukemia, and optionally a pharmacologically accep adjuvant.

According to a preferred embodiment of the compositions according to the

invention the blood drawn from a human suffering from leukemia is first treated in a known manner with an agent known per se against blood clotting.

According to another preferred embodiment of the compositions according to the invention before the removal of the corpuscular elements the blood drawn from a human suffering from leukemia, after an optional treatment with an agent against blood clotting, is subjected to an incubation for decreasing the viability of the leukemic cells.

According to another preferred embodiment of the compositions according to the invention the blood is taken from patients suffering from lymphoid or myeloid leukemia, more preferably in lymphoid leukemia.

The invention relates furthermore to a method for stimulating the immune system which is characterized by administering a patient in need of such a treatment the pharmaceutical composition of the invention in an amount effective for stimulating the immune system.

The method for stimulating the immune system according to the invention can be applied in case of all diseases where this stimulation has a beneficial effects. Without trying to give an exhaustive list of such diseases reference is made to tumors and cancers.

The method for stimulating the immune system according to the invention can be performed prophylactically, therapeutically or post-therapeutically.

According to a preferred embodiment of the method according to the invention the treatment with the composition of the invention is followed by supplementary therapy. This supplementary therapy may comprise the re-administering of blood taken from the patient, administering one or more known vitamins and/or appropriate diet.

As referred to above, in accordance with the present invention blood drawn from patients suffering from lymphoid leukemia and blood drawn from patients suffering from myeloid leukemia can be used but the use of blood drawn from patients suffering from lymphoid leukemia is more preferred. The treatment against

clotting of the blood taken from the patient can be performed by any known method, for example by using sodium citrate, compositions based on heparin, ethylene diamine tetraacetate or by applying any other method described in the publication „Blood transfusion course", published in 1983 by Országos Haematológiai és Vértranszfúziós Intézet (National Hematological and Blood Transfusion Institute) of Budapest, Hungary.

The actual dose of the composition according to the invention can be determined by the physician, depending on the status of the patient. The treatment with the composition according to the invention is carried out suitably for longer periods regularly, preferably two treatments per week are carried out. The preferred weekly dose of the composition according to the invention is 8 ml. The composition according to the invention is administered preferably subcutaneously or intramuscularly.

The invention is illustrated by the following non-limiting examples, wherein reference will be made to the accompanying drawings. In the drawing:

Fig. 1 shows the body mass of BDF_1 mice as a function of time;

Fig. 2 shows tumor volume increase at P-388 leukemia;

Fig. 2a shows comparative data in P-388 tumor volume increase;

Fig. 3 shows tumor volume increase for S-180 sarcoma at mice;

Fig. 3a is a comparative table showing the data of Fig. 3;

Fig. 4 shows the volume increase of S-180 sarcoma;

Fig. 5 is similar to Fig. 4, but has a longer treatment period;

Fig. 6 shows the volume increase of Colon-26 tumor increase;

Fig. 7 is similar to Fig 6, but has a shorter treatment period;

Fig. 7a is a comparative table showing the previous data;

Figs 8 to 28 are different comparative charts showing the number of cells at the control and in case of two dilutions;

Figs 29 to 52 are similar comparative charts to Figs 8 to 28 illustrating the effectivity of the treatment;

Fig. 53 shows the tumor volume in case of S-180 sarcoma;

Fig. 54 is a comparison of the inhibitory effect of treatments of S-180 tumor sarcoma;

Fig. 55 shows the tumor volume in case of P-388 leukemic tumor;

Fig. 56 shows the tumor volume in case of Colon-26 carcinoma;

Fig. 57 shows comparison charts for the previous figure with serum LS;

Fig. 58 shows further comparison charts in case of post-treatment;

Fig. 59 shows further comparison charts for post-treatment with serum LS;

Fig. 60 shows the influence of the serum LS on humoral immune response to SRBC antigen;

Fig. 61 immune response of P-388 s.c. tumor;

Fig. 62 is a chart similar to Fig. 61 taken 8 days after tumor transplantation;

Fig. 63 is a further similar chart for 11 days old tumor;

Fig. 64 is a comparison diagram showing the S-180 sarcoma tumor volume as a function of time;

Fig. 65 is further comparison chart made for S-180 sarcoma;

Fig. 66 shows the tumor volume in case if treatment was applied before tumor transplantation; and

Fig. 67 is a comparison chart in case of P-388 s.c. leukemia.

Example Group I

Results of in vivo human treatments

First the preparation of the compositions according to the invention is shown.

Example A1

About 9 ml of blood drawn for a patient suffering in lymphoid leukemia is

mixed with 1 ml of an aqueous sodium citrate solution of 3 % by weight. The mixture obtained is subjected to centrifuging for 10 minutes in a Rotina 48R centrifuge at 2000 r/m. Three layers are separated, the upper layer and the middle layer with a volume of 7 ml are used furtheron. The composition obtained this way is incubated at +3 °C for 72 hours.

Example A2

40 ml of blood drawn for a patient suffering in lymphoid leukemia is mixed with 2.4 ml of Na-heparin (preparation traded under code ATC-B01AB01 by Gedeon Richter Chemical Works Limited, Budapest, Hungary). The mixture obtained is subjected to centrifuging for 20 minutes in a Rotina 48R centrifuge at 1500 r/m. The upper layer and the middle layer are separated and the composition obtained this way is incubated at +3 °C for 72 hours.

In the following examples the biological effect of the composition according to example A1 is illustrated on the basis of clinical treatments. In these examples the treatment according to the invention means that the patients are treated in the form of a subcutaneous injection weekly two times with 4 ml of the composition according to example A1 each for 4 years and then weekly ones with 8 ml of the composition according to example A1 continuously, depending on the status of the patients.

Example B1

Diagnosis of patient Mrs. B.D. (born in 1947): laryngeal cancer (tumor laryngis supraglottica).

Partial resection of the larynx was performed in 1989 in the Hospital of

Kaposvár, Hungary. The treatment according to the invention was started four weeks after the operation. The patient is free of symptoms and complaints for 9 years.

Example B2

Diagnosis of patient V.Z. (born in 1935): laryngeal cancer (tumor laryngis l.s.).

The treatment according to the invention was started in 1990. The general condition of the patient was good, his body weight did not change for 8 years. He passed away in the autumn of 1998 after an unexpected sudden cardiac arrest.

Example B3

Diagnosis of patient Gy. F. (born in 1957): tumor on the acoustic nerve (tumor nervi acustici l.d.).

The disease started in 1990; symptoms: headache, double vision, partial hearing defect. Surgery was performed in the Department of Neurosurgery of the University of Medical Sciences in Pécs, Hungary, in 1991. Due to the size and location of the tumor only a partial resection was performed. The treatment according to the invention was started in the spring of 1991. Since that time the status of the patient is acceptable, despite the partial surgery there is no deterioration.

Example B4

Diagnosis of patient A.M. (born in 1922): renal tumor (tumor renis).

Surgery was performed in the Department of Urology of the University of Medical Sciences in Pécs, Hungary, in October of 1986 and then in the Department

of Urology of the Hospital of Szeged, Hungary, in March of 1998. The treatment according to the invention was started in December of 1986. The general status of the patient is good, mobile.

Example B5

Diagnosis of patient H.J.: vesical tumor (tumor vesicae urinariae). Result of the histological examination: carcinoma transitiocellulare grad. II. invasive.

Treatment: the disease was diagnosed in connection with haematuria in the Department of Urology of the University of Medical Sciences „Semmelweiss" in Budapest, Hungary, in 1984. Partial removal of the tumor was carried out in two operations, performed in May and June, respectively. Thereafter, in June and July of 1984 cobalt radiation of 48.4 Gy was given. The treatment according to the invention was started in the autumn of 1984. Since that time the general status of the patient is good, no tumor growth could be established.

Example B6

Diagnosis of patient M.F. (born in 1956): testicle cancer (tumor testis). Result of the histological examination: carcinoma embrionale malignus with teratomic parts.

Left-sided testicle and parorchis removal was performed in the Hospital of Zalaegerszeg, Hungary, in May of 1982. Thereafter cytostatic treatment was performed with a combination of vinblastine, cis-platinum and bleomicin. In September of 1982 retroperitoneal lymph node removal was performed. The treatment according to the invention was started in November of 1982. Since that time the general status of the patient is good.

Example B7

Diagnosis of patient P.I. (born in 1933): rectal cancer (adenocarcinoma recti).

The diagnosis was established in February of 1988, then the tumor was removed in the Hospital of Nagykanizsa, Hungary. The treatment according to the invention was started in May of 1988. The patient is free of complaints for more than 10 years.

Example B8

Diagnosis of patient N.F. (born in 1958): rectal cancer (adenocarcinoma recti).

The tumor was removed in the Hospital of Marcali, Hungary, in December of 1991. The treatment according to the invention was started in February of 1992. The weight gain of the patient is 7-8 kg, he is free of complaints and symptoms.

Example B9

Diagnosis of patient Mrs. F.I. (born in 1923): cervical cancer with metastases in the neighborhood. Histology: carcinoma planocellulare.

There was no surgery performed in view of the expanded character of the status. Ra and Tc radiation was performed in the Department pf Obstetry of the University of Medical Sciences in Pécs, Hungary, in August of 1988. The treatment according to the invention was started in October of 1988. The patient was free of complaints for more than 10 years.

Example B10

Diagnosis of patient Mrs. B.D. (born in 1936): uterine body cancer. Histology:

carcinoma corporis uteri.

There was a hysterectomy performed in July of 1985 which was followed by radiation treatment (Ra vaginalis). Cytostatic treatment was performed in the Hospital of Zalaegerszeg, Hungary, in November 1985. The treatment according to the invention was started in January of 1986. The patient was free of complaints for more than 12 years.

Example B11

Diagnosis of patient Mrs. Sz.L. (born in 1904): ovarian cancer with metastases weaving through the entire peritoneum. Result of the histological examination: carcinoma ovarii l.s. inoperabilis. carcinosis peritonei.

The removal of the enlarged tumorous tissue extending above the umbilicus was not possible, therefore no meritorious step was taken. Disclosure of the operations can be found in the Department of Gynecology of the University of Medical Sciences in Pécs, Hungary (September of 1987). The treatment according to the invention was started in November of 1988. The patient was free of complaints and symptoms for more than 11 years.

Example B12

Diagnosis of patient Mrs. Ny. S. (born in 1912): cancer of the stomach. Result of the histological examination: carcinoma cylindrocellulare adenomatosa ventriculi.

A partial resection of the stomach was performed in the National Oncological Institute in Budapest, Hungary, in July of 1966. During the medical examination lymph node metastases were found in the neighborhood. The treatment according to the invention was started in September of 1966. The patient enjoyed weight gain and was free of complaints and symptoms.

Example B13

Diagnosis of patient V. J. (born in 1943): thyroid cancer (adenocarcinoma papillare).

Left-sided thyroid-removal was performed in the Department of Surgery No. II of the University of Medical Sciences in Pécs, Hungary in March of 1991. The treatment according to the invention was started in June of 1991. The patient was free of complaints for more than 7 years.

Example B14

Diagnosis of patient Mrs. M.A. (born in 1936): breast cancer. Histology: ductalis invasive carcinoma.

Right-sided breast and axillary limph node removal was performed in the Department of Surgery of the Hospital of Miskolc, Hungary, in 1991. In November of 1991 radiation treatment was performed. The treatment according to the invention was started in December of 1991. The patient is free of complaints and symptoms since that time.

Example B15

Diagnosis of patient Mrs. G.I. (born in 1924): breast cancer (carcinoma mammae l.s.).

On January 15, 1992 tumor removal and on January 30, 1992 breast and limph node removal were performed. The treatment according to the invention was started in March of 1992. The patient is free of complaints since that time.

Example B16

Diagnosis of patient Mrs. A. I. (born in 1927): breast cancer. Histology: ductalis invasive carcinoma.

Left-sided breast removal was performed in the Department of Surgery of the Hospital of Zalaegerszeg, Hungary, in November of 1985. The treatment according to the invention was started in December of 1985. The patient was free of complaints for more than 13 years.

Example B17

Diagnosis of patient Dr. M.R in the age 50: urinary bladder cancer.

Urological treatment had no result. He treated himself according to the invention. First treatment was performed for 5 years as of 1954. He was free of symptoms for 38 years. After having very same symptoms the treatment according to the invention was re-started in November of 1998. The patient's status is continuously improving.

In the following examples the biological effect of the composition according to example A2 is illustrated on the basis of clinical treatments. In these examples the treatment according to the invention means that the patients are treated in the form of a subcutaneous injection once in a week with 8 ml of the composition according to example A2.

Example C1

Diagnosis of patient L.L. (born in 1931): bladder cancer.

Partial tumor removal through the urethra was performed several times in the Department of Urology of the Hospital of Zalaegerszeg, Hungary, between 1991 and 1997. The treatment according to the invention was started in September of 1998. The patient is free of complaints and symptoms.

Example C2

Diagnosis of patient T.T. (born in 1940): laryngeal cancer (tumor laryngis supraglottica).

After medical examination patient refused surgical treatment. The treatment according to the invention was started in January of 1997. The patient is free of complaints and symptoms.

Example C3

Diagnosis of patient Mrs. R.J. (born in 1923): rectal cancer. Histology: adenocarcinoma recti.

After radiation treatment rectum removal by surgery was performed in 1995 which was followed by a treatment with a cytostatic agent. The treatment according to the invention was started in July of 1997. The patient is free of complaints and symptoms.

Example C4

Diagnosis of patient S.I. (born in 1939): cancer of the stomach. Histology: anaplasticus adenocarcinoma.

Complete gastrectomy was performed in the Department of Surgery of the Hospital of Zalaegerszeg, Hungary, in May of 1997. The treatment according to the invention was started in July of 1997. The patient is free of complaints and symptoms.

Example C5

Diagnosis of patient Mrs. T.I. (born in 1924): breast cancer. Histology:

infiltrative ductalis carcinoma.

Breast and axillary lymph node removal was performed in the Department of Surgery of the Hospital of Szeged, Hungary, in March of 1994. The treatment according to the invention was started in July of 1994. The patient is free of complaints and symptoms.

Example C6

Diagnosis of patient U.I. (born in 1931): vocal cord tumor. Histology: carcinoma planocellulare.

Radiation treatment was performed in the Department of Otorhinolaryngology of the University of Medical Sciences „Semmelweiss" in Budapest, Hungary, in January of 1994. The treatment according to the invention was started in February of 1994. The patient is free of complaints and symptoms.

Example C7

Diagnosis of patient F.L. (born in 1927): pancreas cancer.

In view of the type of the tumor no treatment was performed. During medical examination the presence of a metastasis in the left-sided adrenal gland was verified. The treatment according to the invention was started in January of 1996. The patient is free of complaints and symptoms.

Example C8

Diagnosis of patient Mrs. P.F. (born in 1951): breast cancer. Histology: carcinoma lobulare mammae.

Removal of tumor from the left-sided breast and axillary lymph node removal was performed in the Department of Surgery of the Hospital of Kaposvár, Hungary,

in September of 1994. Radiation treatment was performed in the Department of Radiology of the University of Medical Sciences in Pécs, Hungary, in January of 1995. The treatment according to the invention was started in February of 1995. The patient is free of complaints and symptoms.

Example C9

Diagnosis of patient Mrs. B.L. (born in 1939): colon cancer.

Removal of tumor was performed in the Department of Surgery of the Hospital of Kaposvár, Hungary, in 1991. Radiation treatment was performed in the National Oncology Institute in Budapest, Hungary, in 1992. In 1995 a recurrent tumor was surgically removed. The treatment according to the invention was started in 1995. The weight gain of the patient was 6 kg, she is free of complaints and symptoms.

Example C10

Diagnosis of patient K. Gy. (born in 1911): prostate cancer.

Surgical removal was performed in the Department of Urology of the Hospital of Kaposvár, Hungary, in 1993. The treatment according to the invention was started in 1994. The patient is free of complaints and symptoms.

Example C11

Diagnosis of patient Cs. A. (born in 1942): liver cancer.

In view of the type of the tumor no surgery was performed. The treatment according to the invention was started in March of 1997. An ultrasound control of the abdomen, performed in the clinic of Balatonfüred, Hungary, in September 1997, verified regression.

The above test examples verify the usability of the compositions according to the invention for treating tumorous diseases and preventing the formation of new tumors.

Example Group II

Report on experiments made on experimental animals and cell cultures

In this example group four types of preparations were made from the blood samples taken from persons suffering in leukemia. In all the three preparations the so obtained leukemic blood was the starting material and no anti-coagulant was added. The blood was cooled down to about 4°C after about 5 to 10 minutes it was sampled, and after a storage of 24 to 48 hour5s it was treated as follows.

Preparation of leukemic serum (LS). The cooled starting blood was centrifuged with 1500g for a period of 10 minutes. The temperature was between 4°C and 10°C. The machine used was the type Janetzky K26. The rotor was angular one. The serum obtained was the upper liquid phase. Up to its actual use it was stored in frozen state. In use a dose of 0.2 ml/mouse was applied.

Preparation of leukemic blood (LB). The starting material was diluted by 2.5 amount of physiologic saline solution (0.9% NaCl). Dosage: during the experiments a dose of 0.2 ml/mouse was applied. This dose is about 50 times as high as the human dose when calculated to a unity weight.

Preparation of lyophilized blood (LyB). The starting material was lyophilized in a freezing-drying machine having the type of LGA 05 made by MLV Labortechnik, Ilmenau, Germany. The temperature was –205C, pressure somewhat below atmospheric one. Time of exposure 16 to 20 hours. Yield: from a starting blood of 1 ml about 300 mg LyB was obtained.

Preparation of leukemic blood extract (LE). 10 ml of the starting blood was centrifuged with 1500g for 10 minutes with the same machine as used for the

material LS, the temperature was 4°C. An extracting solution of 3 ml and consisting of 0.4 M NaCl, 0.01 M Tris HCl and 0.001 M EDTA buffer with a pH 8.2 was added to the sediment. After a storage of 16 hours a further centrifugation was applied with 1000g using the same machine. The upper liquid component was taken. In use the liquid was diluted in 3:1 ratio with a physiologic saline solution. The dose was 0.2 ml/mouse.

The animals used were mice of the following types:

BDF_1: Specified pathogen free inbred hybrid mice coming from C57 B1/KalWrij female x DBA/2 LWM61Rij male from the TNO Inst. Applied Radiobiol. Immunol. Rijswijk, Netherlad.

Balb/c: Specified Pathogen Free inbred mice coming from Balb/c AnCrlRij, from the same Institute.

Experiment series 1 (2. jelentés)

a) Acute toxicology tests

The mice were given 0.2 ml of the preparation LB in subcutane and intra peritonial every second each day two or three times. In an experimental period of 21 days all animals survived. Fig. 1 shows the increase in weight of groups each consisting of 10 mice during the experimental period. The weight versus time curves have demonstrated that the treatments have not caused any loss in weight that would indicate a toxic effect. The curves at the treated group were equal to that of the control group.

b) Survival and tumor size experiments in case of induced P-388 lymphoid leukemia

The implanted tumor was P-388 lymphoid leukemia coming from Arthur

Little and Co., Cambridge, MA, USA. The transplantation of the tumor was at the first series intraperitoneal, so called ascites tumor, and in the second series it was subcutane dosed, so called solid tumor. The given dose was 1 $x10^7$/mouse. The mice used were BDF_1 type.

Table 1 shows the survival data in case of P-388 ascites tumor. The preparation LS caused a more than 20% increase in survival, while the preparation LB was very close to 20%, which amount is considered by the NCI (National Cancer Institute, USA) as an acceptable limit value. The preparation LyB did not cause a significant increase in survival.

Table 2. shows the survival data in case of subcutane implanted P-388 leukemia. Here the preparations LB and LyB did not give a significant survival.

Table 1

Test groups	Type of treatment	Frequency of treatment	Number of test animals	Average survival (days)	Survival over control %
LB	s.c.	2 x q3d	5	15.2 ± 2.4	119
LS	s.c.	2 x q3d	5	15.6 ± 4.2	122
LyB	s.c.	2 x q3d	5	14.0 ± 1.2	109
K (P-388, i.p.)	-	-	5	12.8 ± 1.09	100

First treatment: 3 days after tumor implantation

Table 2

Test groups	Type of treatment	Frequency of treatment	Number of test animals	Average survival (days)	Survival over control %
LB	s.c.	5 x q2d	7	22.4 ± 2.6	112
LyB	s.c.	3 x q2d	7	21.9 ± 0.9	110
K (P-388, s.c.)	-	-	7	20.0 ± 2.3	100

First treatment: 2 days after tumor implantation

The volume of the tumor was calculated by the following formula:

$$V = \pi/6 \times L \times D^2$$

where V is the tumor volume, L is the longest diameter and D is the shorter diameter normal to the longest one.

Figures 2 and 2a show the results of the volume measurements. The five treatments with the preparation LB provided a nearly 60% reduction in tumor volume, while the three treatments with the preparation LyB resulted in a 30% volume reduction.

c) Survival and tumor size experiments in case of S-180 sarcoma

The S-180 sarcoma was obtained from Chester Beatty Cancer Res. Inst., London. It was transplanted in subcutane manner into BDF_1 mice.

Tables 3, 4 and 5 summarize the results of the survival experiments carried out with the preparations LB, LS and LyB, respectively. The animals were treated as given in the tables.

Table 3

Test groups	Type of treatment	Frequency of treatment	Number of test animals	Average survival (days)	Survival over control %
LB	s.c.	5 x q2d	5	24.0 ± 4.5	105
LS	s.c.	5 x q2d	5	23.0 ± 2.6	101
LE	s.c.	5 x q2d	5	23.8 ± 3.0	104
K (Colon-26)	-	-	5	22.8 ± 2.5	100

First treatment: 2 days after tumor implantation

Table 4

Test groups	Type of treatment	Frequency of treatment	Number of test animals	Average survival (days)	Survival over control %
LS	s.c.	8 x q2d	5	23.5 ± 472	104
K (S-180)	-	-	5	22.6 ± 3.3	100

First treatment: 2 days after tumor implantation

Table 5

Test groups	Type of treatment	Frequency of treatment	Number of test animals	Average survival (days)	Survival over control %
LB	s.c.	10 x q2d	5	23.8 ± 2.2	104
LE*	s.c.	10 x q2d	5	24.2 ± 2.1	105
K (S-180)	-	-	5	23.0 ± 1.4	100

First treatment: 2 days after tumor implantation

* First treatment: 4 day after tumor implantation

In the survival tests no significant increase was obtained in any of the treatments.

The tumor volume curves of Fig. 3 and 3a demonstrate, however, a significant volume inhibition effect.

The treatment was terminated on the 9th day. On the 11th day the preparation LS was the most efficient with an inhibition effect of 65%. The other two preparation provided a more than 50% inhibition. The inhibition effect was influenced by the time of treatment. It can well be seen that after 6 days following the termination of the treatment the tumor inhibiting effect decreased.

Figs. 4 and 5 show the volume in case of longer treatments. Fig. 5 shows that the tumor inhibition effect is higher at the early stage of treatment than at a later phase.

d) Survival and tumor size experiments in case of Colon 26 type colon carcinoma

The mice were given Colon-26 type colon adeno carcinoma obtained from SRI, Birmingham, Alabama, USA. The was of transplantation was subcutane.

The results of the survival experiments are summarized in Tables 6 and 7.

Table 6

Test groups	Type of treatment	Frequency of treatment	Number of test animals	Average survival (days)	Survival over control %
LB	s.c.	10 x q2d	5	36.1 ± 1.9	117
LS	s.c.	7 x q2d	5	35.4 ± 3.1	114
K (Colon-26)	-	-	5	31.0 ± 1.9	100

First treatment: 2 days after tumor implantation

Table 7

Test groups	Type of treatment	Frequency of treatment	Number of test animals	Average survival (days)	Survival over control %
LS	s.c.	8 x q2d	5	30,34,36,42>52	
LE	s.c.	8 x q2d	5	35.8 ± 5.61	105
K (Colon-26)	-	-	5	32.6 ± 3.8	100

First treatment: 2 days after tumor implantation

The survival data were better than in case of the previous two subcutane experiments. From the group treated with the preparation LS an animal was still alive at the time of writing the present report, and it means a 60% increase in survival time relative to the control group.

The tumor volume curves are shown in Figs. 6 and 7, that demonstrate the favorable volume inhibition effect in case of all the three preparations. The figures also show that the inhibition effect increases with the number of treatments.

Experiment series 2

In vitro experiments 1st part

The tumor inhibiting effects of the preparations LyB, LS and LE described in the previous chapter have now been examined on the basis of in vitro experiments. The tumor cells were grown in a medium of RPMI 1640 that comprised 10% of fetal calf serum. The cultures were treated through 24 hours by different concentrations of the preparations LyB, LS and LE. The concentration values expressed in microgram/ml, and the amount of dilution were always indicated in the results.

a) examination of cytotoxic effect on a fibroblast cell line of mice

Figures 8 to 10 show the results obtained by the preparations applied in four different doses and two dilutions. The results demonstrate that the preparations, even in the applied high doses were not toxic relative to the healthy cells.

b) Examination of anti tumor effects in different kinds of mice and human cancer cell strains

The following cell lines were examined:

- MDA-MB-231. This is a human breast adeno carcinoma cell strain growing in monolayers (oestrogen receptor is negative, ER-)
- MCF-7. This is a human breast adeno carcinoma cell strain growing in monolayers (oestrogen receptor is positive, ER+)
- HT-29. This is a human colon carcinoma cell strain that grows in a suspension.
- C-26. This is a colon carcinoma cell strain of mice.
- K-562. This is a human leukemia cell strain.
- PC-3. This is a human prostate carcinoma cell strain.

Results of the experimental examination

Figs 11 to 16 show the effects of the preparation LyB given in four different doses (i.e. 120, 240, 480 and 960 μg/ml) on the growth of the listed types of tumor cells.

In a dose of 960 μg/ml the preparation LyB decreased the viability of the human tumor cells MDA-MB-231, PC-3 and of the mice tumor cells C-26 by about 35-40%. It had only a limited effect on the human tumor cells MCF-7 and HT-29 (about 25%). A cell decrease of about 20% was obtained in case of the K-562 leukemia culture. The effect was dependent on the dose in case of all kinds of tumors tested.

Figs. 17-22 show the effects of the preparation LE in two concentrations. The preparation LE proved to be more efficient in the lower concentration. Against the human tumor cells MDA-MB-231 and PC-3 a decreasing effect of 40% was attained. In case of the other cell cultures the effect was less than 20%.

Figs. 23 to 28 show the effects on the viability of the listed tumor cell cultures

of the preparation LS. Of these cultures an inhibition effect was experienced in case of the cells PC-3 (36%) and of the cells MDA-MB-231 (30%). This preparation, similar to the previous one, was more effective in the lower concentration.

Experiment series 3 (

In vitro experiments 2nd part

At a later date, in vitro tests were carried out using the same cell lines as in the Experiment series 2 (1st part of the in vitro tests).

All the cell lines were cultured in RPMI 1640 medium (Sigma) supplemented with 10% fetal or newborn calf serum. Subcultivation was made twice a week. For determining of surviving fraction, exponentially growing cells were plated at a density of 0.3 to 0.5 x 10^6 cells.

After 24 hours of the treatment with various concentrations of leukemia blood preparations the cells were stained with 0.1% Trypan blue and counted by a hematocytometer.

The tests were carried out with the preparations LS and LE. In this experiments the preparation LS (leukemic serum) was slightly different from that described in the previous example. The whole blood taken from leukemia donors was kept at 4°°C for four hours and was centrifugated at 4000g. The serum LS was the liquid fraction.

As a control normal serum NS was examined taken from healthy persons and obtained in the same way as the serum LS.

Results and discussion.

a) Effects on the growth of MDAMB 231 breast carcinoma cells

The experiments have shown that normal serum NS increased the proliferation of MDAMB 231 breast carcinoma cells by 2-3% as shown in Fig. 29). At the same

time 1/10 and 1/20 dilution of leukemia serum LS decreased the growth of the same type of cells by 42% and 30%, respectively (Figure 30).

b) Effects on the growth of MCF-7 breast carcinoma cells

The normal serum NS had no effect on the growth of MCF-7 breast carcinoma cells as shown in Figure 31). These cells contain estrogen receptors ER. The ER+ MCF-7 cell line is less sensitive to the serum LS than the ER- type MDAMB-231 cell line. The 1/10 and 1/20 dilution of leukemia serum inhibited the cell proliferation by 22% and 17%, respectively (Figure 32). The anticancer activity of leukemia serum LS has been confirmed in the case of MCF-7 cell culture, as well.

c) Effects on the growth of HT 29 colon carcinoma cells.

The effect of serum NS on the growth of HT 29 colon carcinoma cell is shown in Figure 33. The normal serum NS induced a slight increase in the proliferation of HT 29 colon carcinoma cell culture. At the same time, the leukemia serum LS decreased the growth of HT 29 colon tumor cells by 1926% (Figure 34).

Our studies have shown that the leukemia serum is able to inhibit the proliferation of HT 29 colon carcinoma cells, as well.

d) The effects of normal serum NS) and leukemia serum LS on the growth of C 26 colon carcinoma cells.

The effects of normal serum NS on the proliferation of C 26 colon carcinoma cells is demonstrated in Figure 35. This figure shows that the serum NS did not inhibit but increased the growth of C 26 colon carcinoma cells. On the other hand, treatment with serum LS in 1/10 or 1/20 dilution diminished the cell number by 38-12%, respectively (Figure 36).

Our studies have confirmed that the leukemia serum LS has anticancer activity.

e) The effect of normal serum NS and leukemia serum LS)on the growth of K 562 leukemia cells.

The data presented in Figure 37 clearly demonstrate that normal serum NS caused no decrease in the proliferation of K 562 leukemia. A significant decline (36%) in the cell proliferation of K 562 leukemia could be observed after treatment with leukemia serum LS (Figure 38).

Our studies revealed that leukemia serum LS is able to inhibit cell proliferation of heterologuous leukemia cells.

f) The effect normal serum NS and leukemia serum LE on the growthof PC 3 prostate cancer cells.

Various dilutions of normal serum NS induced a slight stimulation in the tissue culture of PC 3 prostate cancer cells (Figure 39). However, leukemia serum LS resulted in a 36-38% decline in the cell number of PC 3 prostate cancer (Figure 40).

Our results suggest that PC 3 prostate cancer cells have a high sensitivity to the growth-inhibitory effect of leukemia serum.

g) The effect of normal blood extract (NE) and leukemia blood extract onthe MDAMB 231 breast cancer cells.

The effect of serum NE on the growth of MDAMB 231 breast cancer cells is shown on Figure 41. There is no decrease in the cell number of MDAMB 231 cell culture after treatment with NE (Figure 42).It should be noted that 1/10 dilution of

leukemia blood extract LE inhibited the proliferation of MDAMB 231 cell line by 45% (Figure 42).

Our results suggest that cellular components of blood might synthesize biologically active macromolecules with antitumor effects.

h) The effect of normal blood extract NE and leukemia blood extract LE on MCF-7 breast carcinoma cells.

Figure 43 shows the effect of NE on MCF-7 breast carcinoma cells. NE treatment caused no decrease in the proliferation of MCF-7 cell line.

At the same time LE treatment induced 18-22% of cell death in MCF-7 cell culture (Figure 44).

The growth inhibitory effect of LE is similar to that of LS in the case of MCF-7 breast carcinoma cells.

i) The effect of normal blood extract NE and leukemia blood extract on the growth of HT 29 colon carcinoma cells.

The treatment with serum NE resulted in no decrease in the growth of HT 29 colon tumor culture (Figure 45). However serum LE treatment inhibited the cell proliferation of HT 29 cell culture by 19-34% (Figure 46).The antitumor activity of LE is more pronounced than that of the leukemia serum (LS).

j) The effect of normal blood extract NE and leukemia blood extract LE on C 26 colon carcinoma cells.

The effect of extract NE on the growth of C 26 colon tumor cells is summarized in Figure 47. A moderate growth stimulation could be observed in the NE treated culture of C 26 colon carcinoma. The growth of C 26 colon cells is inhibited by 14-41 % after treatment with leukemia blood extract (Figure 48).

The growth-inhibitory effect of the extract LE is greater than that of the leukemia serum. This finding lends further support to the idea that the anti tumorous

macromolecules are synthesized by the cellular components of the blood.

k) The effect of normal blood extract and leukemia blood extract on K 562 leukemia.

The growth of K 562 leukemia is stimulated moderately by the treatment of NE (Figure 49). At the same time the leukemia blood extract cause a 1840% decrease in the proliferation of K 562 cells (Figure 50).

The antitumor effect of LE is higher than that of the LS.

l). The effect of normal blood extract NE and leukemia blood extract on the PC 3 prostate tumor cells.

Treatment of PC 3 prostate cancer cells with the extract NE caused no decrease in their proliferation (Figure 51).

On the other hand LE treatment resulted in 40-42% decrease in the cell number of PC 3 prostate cancer (Figure 52).

Our results lend further support to the idea that the leukemia blood extract LE contains higher level of macromolecules with antitumor activity than that of the leuke-mia serum.

Experiment series 4.

Comparative in vivo Examinations

Aim of study:

* Influence of the sera derived from human leukemic patients and healthy donor on the growth of different type transplantable rodent tumors. Comparative in vivo investigation.

* Effect of leukemic- and normal serum on the immune response to sheep red blood cell antigens in healthy and tumor bearing mice.

Materials and methods:

The following preparations were used: serum from leukemic blood LS; extract preparation LE; serum from healthy donors, i.e. normal serum NS

The experimental animals were mice: BDF_1 (inbred) first generation hybrid mice and Balb/c inbred mice weighing 21-22 g from our specified pathogen free (SPF) breeding.

The animals were kept in macrolon cages at 23-25°C (40-50% humidity), with a lighting regimen of 12/12 h light/dark. The animals had free access to tap water and were fed with a sterilized standard diet (Altromin 1324 pellets, Altromin Ltd, Germany) ad libitum.

Table 8 Rodent tumor models

Tumor code	Tumor type	Animal strain	Inoculum	Graft route	Origin
P-388	lymphoid leukemia	BDF_1	1×10^7 cells	s.c.	Cambridge MA, USA
S-180	sarcoma	BDF_1	fragment	s.c.	Chester Beatty Inst. London
Colon-26	carcinoma	Balb/c	fragment	s.c.	SRI Birmingham Alabama, USA

Doses and treatment schedule

- Doses of sera (LS or NS) : after centrifugation of blood the supernatant (serum) was applied. Dose : 0.2 or 0.3 ml sera/mouse
- Dose of blood-extract (LE) : after the lysis and centrifugation of blood, supernatant was diluted to its three fold. Dose : 0.2 ml sup.nat. per mouse

The treatments were started before or after the transplantation of tumor and repeated 4, 5, 6 times.

Methods for evaluation

Antitumor activity

Repeated treatments were started several days before or on day 1 after transplantation of P-388, S-180 or Colon-26 tumors.

The therapeutic effectiveness was evaluated on the basis of survival time and tumor volume.

The comparative antitumor effect of various treatment groups on the median survival time in days for treated versus control groups was expressed as T/C.

According to the screening program of NCI (National Cancer Institute, USA) the minimal criterion for acceptable activity of a drug increase the life span with 20% (Staquet M.I., Byar D. et al. Clinical predictivity of transplantable tumor systems in the selection of new drugs for solid tumors: Rationale for a three-stage strategy. *Cancer Treat.Rep.*, 67, 753-765, 1983). Where the T/C is the ratio of the median survival time of the treated group over that of the untreated control group; minimal effectiveness: $T/C \times 100 \geq 120\%$.

With the P-388, S-180 and Colon-26 solid tumors the tumor growth inhibitory effect of sera was controlled 3 times a week using digital caliper. Tumor volume was calculated using the following formula:

$$V = a^2 \times b \times \pi/6$$

where "a" and "b" mean the shortest and longest diameter, respectively of a given tumor (Tomayko M.M. and Reynolds C.P.: Determination of subcutaneous tumor size in athymic /nude/ mice. *Cancer Chemother. Pharmacol*, 24, p.148, 1989).

Mean values (X) and standard deviations (S.D.) were calculated.

Investigation of the humoral immune response

The haematological plaque-forming cell (PFC) assay first described by Jerne and Nordin (Jerne N.K., Nordin A.A., *Science*, **140,** 405, 1963) was adapted by us (Gaál D. and Nowotny A., *Cancer Immunol. Immunother.*, 6, 9-15, 1979). With this modified procedure it is possible to determine how many of the total cells present in spleen are producing and secreting antibodies.

Sheep red blood cells (SRBC) were used as antigens for immunization.

The PFC assay was carried out 4 days after immunization with SRBC antigen according to the schematic outline.

Evaluation

Count the plaques per plate. Compute the number of antibody-producing cells per 10^6 spleen cells. Compare the findings with the control group.

The immuno modulatory effect of treated groups was expressed in the percent of control.

Results and evaluation

a. Influence of leukemic serum (LS) on the growth of S-180 sarcoma

Treatments <u>before</u> tumor transplantation

The tumor inhibitory effect of leukemic serum applied in different doses and treatment schedule (6×q2d and 4×q3d) was studied on S-180 sarcoma.

The leukemic serum applied 4 or 6 times before tumor transplantation inhibited the tumor growth in 40%. The inhibition increased parallel with the tumor

development (see *Figure 53* and 57).

b. Comparative studies on the tumor growth inhibitory effect of the sera from leukemic patients (LS) and healthy donor (NS) on the growth of transplantable tumors

b.1. In S-180 sarcoma

The effects of leukemic serum LS and normal serum NS applied after tumor transplantation were compared with one another on the S-180 tumor (*Figure 54*).

The serum derived from the healthy donor did not influence the growth of tumor.

However, the leukemic serum injected five times after tumor transplantation evoked a significant inhibitory effect. The mean of tumor volumes was only 50% as compared to the mean volumes of untreated group, as shown in *Figure 57* (second and third columns).

b.2. In P-388 lymphoid leukemia

The tumor inhibitory effect of the leukemic serum LS was most evident when it was given 5 times beginning 1 day after tumor inoculation (*Figure 55*).

Figure 58. shows that 80% of tumors were inhibited by the leukemic serum LS as compared to that of untreated control.

The tumor inhibitory effect of serum from the healthy donor was practically negligible.

b.3. In Colon-26 tumor

As shown in *Figure 56,* the normal serum NS practically did not influence the

growth of tumor. However, the tumor inhibitory effect produced by the leukemic serum LS was more than 40% on 14 days after tumor transplantation and 3 days after the last treatment (see *Figure 59*).

c) The influence of leukemic (LS) and normal (NS) sera on the survival of transplantable tumors, different types

On the basis of mean survival times a similar ineffectivity of leukemic and normal sera was observed in mice bearing S-180 and P-388 (s.c.) tumors irrespective of treatment schedule (pre-/ or post-treatments) demonstrated in *Tables 9.* and 10.

As shown in *Table 11*, the leukemic serum LS was able to increase the survival of Colon-26 tumor bearing mice in a measure, specified by the NCI (T/C × 100 > 120%, Staquet M.I., Byar D. et al., *Cancer Treat.Rep.*, 67, 753-765, 1983).

Table 10

Effect of sera derived from leukemic and normal blood on the survival of P-388 leukemia tumor bearing mice

Treatment groups	*Dose ml/mouse*	*Treatment*		*No. of animals*	*Mean survival (days) average X, S.D.*	*Survival T/C%*
		time	*schedule*			
LS	0.2	*post-treatment	5 × q2d	7	20.9 ± 2.9	102
NS	0.2		5 × q2d		20.6 ± 2.6	101
Control	-	-	-		20.4 ± 3.3	100

**Start of treatments: 1 day after tumor transplantation*

LS = leukemic serum

NS = normal serum

Control = untreated control

immune response to SRBC antigens in the LS-treated healthy mice was nearly 60%. This is an advantageous feature of the leukemic serum, since the tumors generally evoke an immuno suppression.

Further experiments on the time-dependent effect of leukemic serum on the humoral immune response to SRBC antigens in healthy BDF_1 mice and/or in tumor bearing mice are in progress.

Summary of this test series

1. Pre-treatments with serum derived from the blood of leukemic patients (repeated treatments before tumor transplanation) induced 40% tumor growth inhibitory effect against S-180 sarcoma. The inhibitory effect of leukemic serum increased parallel with the development of tumor (see *Figures 53* and 57).

2. The growth and development of different tumors were inhibited by leukemic serum in 40-80% depending on the type of the tumor (see *Figures 54, 55, 56, 57, 58* and *59*). The P-388 lymphoid leukemic tumor proved to be the most sensitive against leukemic serum. The post-treatments by leukemic serum produced nearly 80% growth inhibition (*Figure 58*).

3. Data of our comparative investigations showed that the serum derived from a healthy donor practically did not influence the growth of different type rodent tumors (see *Figures 54, 55, 56* and 58, 59).

4. The leukemic serum was able to increase the survival of Colon-26 tumor bearing mice to an extent (specified by the NCI, *Table 11*) same to our earlier results (see the previous report, No. 2629/1998).

response of the tumorous host was compensated in the tumor bearing mice pretreated (2×) with leukamic serum (see *Figure 61*)

A significant immunosuppression (over 50% inhibitory effect of the immune response) was observed in the developed tumor bearing mice (on the 8th day after tumor transplantation).

This considerable inhibitory effect on the humoral immune response was compensated completely by the leukemic serum (*Figure 62*).

At an advanced stage of tumor development (on the 11thday after tumor transplantation) a similarly significant immunosuppression was observed in the 11 days old tumor bearing animals.

The leukemic serum injected on days 14 and 18 before PFC assay reduced the tumor-induced significant immunosuppression to its half value (*Figure 63*).

Summarizing our experimental data it is established that the leukemic serum LS having an immunoadjuvant potential could advantageously influence the suppressed immune response of the tumor bearing host (*Table 12*).

A : normal (SRBC) control

B: tumorous control
- 4 days-old tumor (I. exp)
- 8 days-old tumor (II. exp.)
- 11 days-old tumor (III. exp.)

C: LS-treated tumor bearing mice

Table 13

Influence of the leukaemic serum and the extract from leukaemic blood on the survival of S-180 sarcoma bearing mice

Treatment	*Dose ml/mouse*	*Treatment*		*No. of animals*	*Survival time (days) average X S.D.*	*Survival T/C%*
		time	*schedule*			
LS	0.2	*pretreatment	4 × q2d	7	26.6 ± 3.7	125
NS	0.2		4 × q2d	6	25.8 ± 7.08	121
Control	-	-	-	7	21.3 ± 1.5	100

**pretreatment: after tumor translplantation, every two days (q2d)*

LS = leukaemic serum

NS = extract from leukaemic blood

Control = untreated tumor bearing mice

The leukemic serum produced nearly 40% tumor growth inhibition, and this proved to be more effective than the effect of blood-extract LE.

As shown in *Table 13*, the preparations LS and LE were able to increase the mean survival of S-180 tumor bearing mice in a relevant extent specified by the NCI (T/C × 100 > 120%, Staquet M.I., Byar D. et al., *Cancer Treat.Rep.*, 67, 753-765, 1983) same to previous results on Colon-26 tumor.

c) Influence of leukemic serum LS on the growth of P-388 s.c. lymphoid leukemia and on the survival of tumor bearing mice

The tumor inhibitory effect of leukemic serum LS applied in pretreatments (2×) is demonstrated in *Figures 66* and 67

The retardation effect of tumor development and a significant tumor growth inhibitory effect of leukemic serum were observed.

On day 13 after tumor transplantation the tumor inhibition was over 60% (*Figure 67*).

As shown in *Table 14*, the mean survival of LS-pretreated tumor bearing mice did not reach the measure of efficacy expected by NCI (T/C × 100 > 120%).

III. Examination Group

Experiments with enzymatic tumor markers with induced mammal tumors in rabbits

Enzymatic tumor markers are widely used in experiments directed to monitor the progress of tumors, which reflect metabolic changes that can be characteristic to the malignant processes. We have selected three metabolic characteristics, namely the serum 5'-nucleotidase activity, the serum arginase and the serum ornintine-carbamyl-transferase. Of these parameters the first one, i.e. the serum 5'-nucleotidase

rised lipids and which was free of lipids. The fraction that comprised lipids was diluted to take the original volume by the addition of a physiologic saline solution, and this formed the 1st experimental material. The lipid-free second fraction formed the 2nd experimental material.

The experiments were carried out with matured female New-Zealand rabbits. First a preliminary experiment series were carried out using respective single animals in each experimental category.

In a control animal no treatment was carried out, only test measurements were carried out at the three given dates, i.e. on 6th, 13th and 19th December 1999. In case of three animals breast tumor cells were injected in the breast on 2nd December. Two of the three experimental animals were treated by the 1st and 2nd experimental materials, respectively on the day of the tumor implantation and on the days when the tests were carried out. Each treatment comprised 0.6 ml of the experimental material applied in the form of intramuscular injection. The third animal was not treated, it constituted the positive control. By 13th December it was clear that the so designed test has proven to be informative, and a further experiment with respective four rabbits in each of the three groups were used. These rabbits were implanted with the same tumor in the same way on 13rd December, and the animals in the first two groups were treated with the 1st and 2nd experimental materials. Tests were made on 19th December, 1999 and thereafter in weekly periods, but for the time being only the results from 19th December are available.

The results of the tests are summarized in 15 to 17, respectively. In case of experiments with four animals in each group the results given are the averages of the individual measured values.

Values of serum arginase U/I

Table 16

	Preliminary experiment			
Date	control without tumor implantation	preliminary experiment 1st material	preliminary experiment 2nd material	preliminary experiment positive control
December 06, 1999	8.11	7.98	9.12	10.45
December 13, 1999	8.11	6.56	5.76	38.56
December 19, 1999	8.11	10.08	13.9	56.7

	Experiments, averages of 4 mice		
Date	101-102 positive control	103-104 1st material	105-106 2nd material
December 19, 1999	35.2	16.7	8.2

control group an increase was experienced. These two tests together make it very probably that the values of the total serum 5'-nucleotidase activities express the progress of the tumor.

While in case of the positive control both in the preliminary experiment and in the experiment this value increased substantially, the treated animals either retained the normal value, or it decreased to a negative one in case of the 1st test material (that comprises lipids). In case of the 2nd test material, in the preliminary experiment on 13rd December the value increased, but the increased value was still about the half of the positive control. In 6 days, this value returned to normal, while the positive control was still high.

These tests have also demonstrated that leukemic blood comprises components, which have a strong tumor depressive effect.

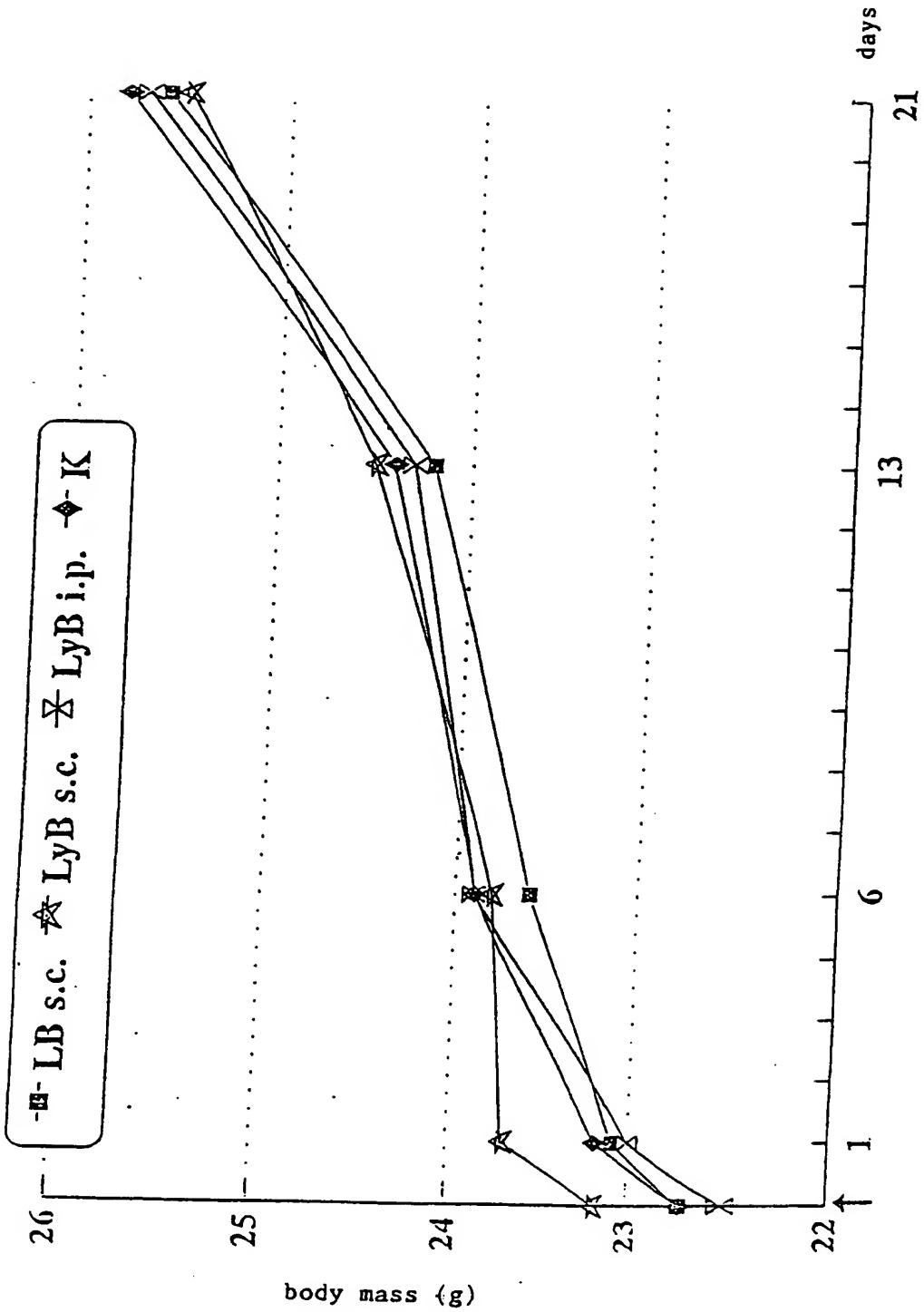

Fig. 1

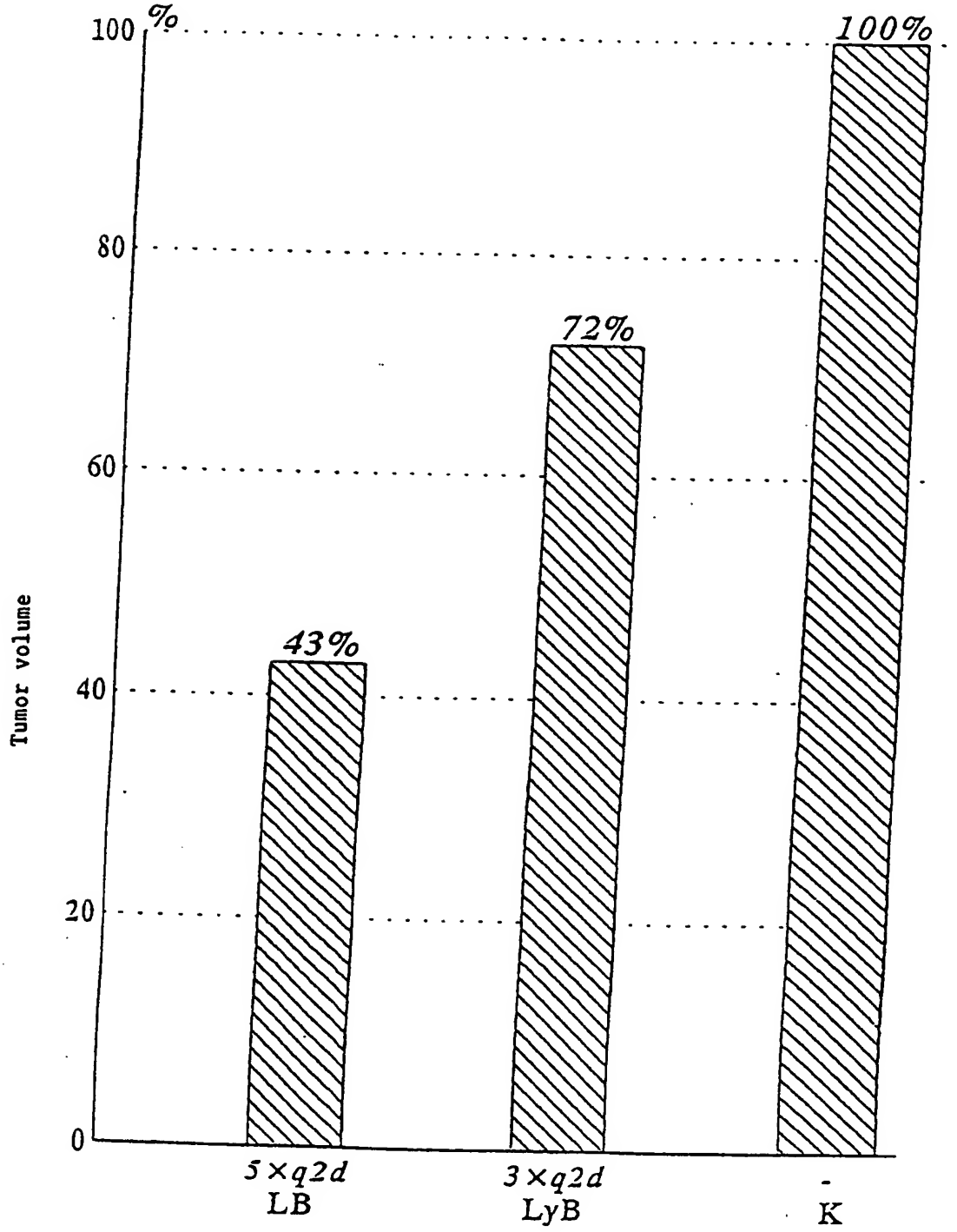

Fig. 2a

P-388

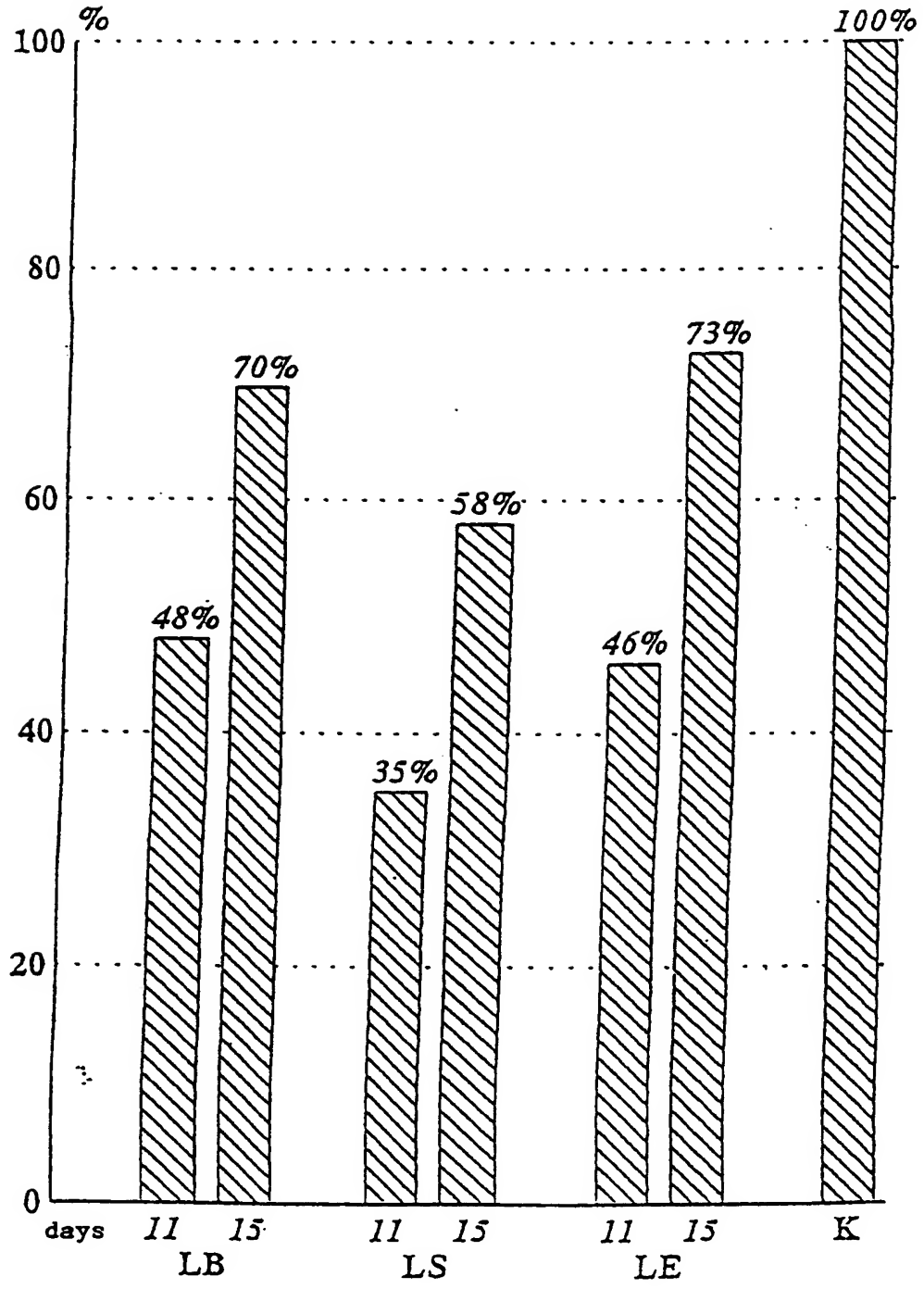

Fig. 3a

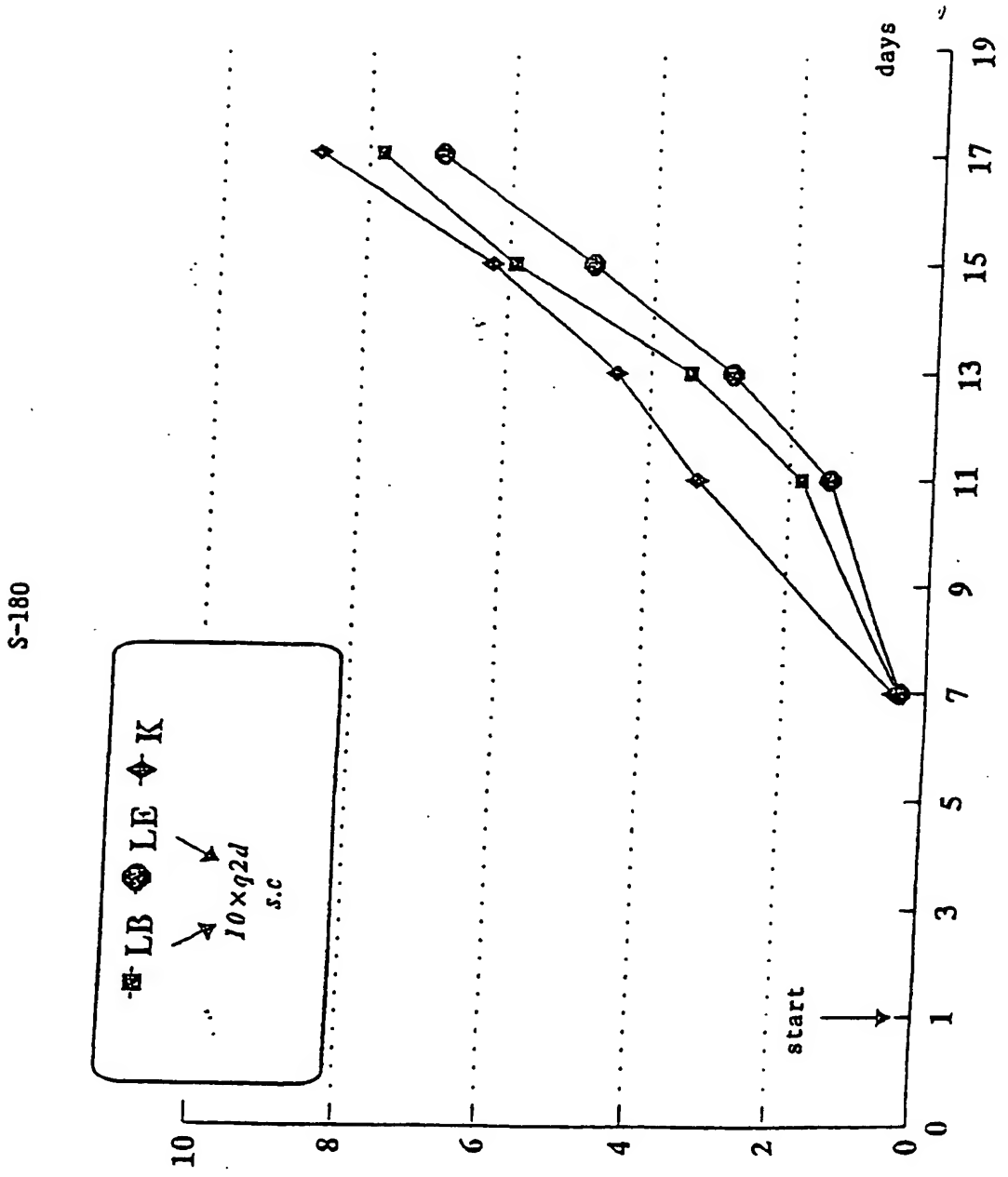

Fig. 5

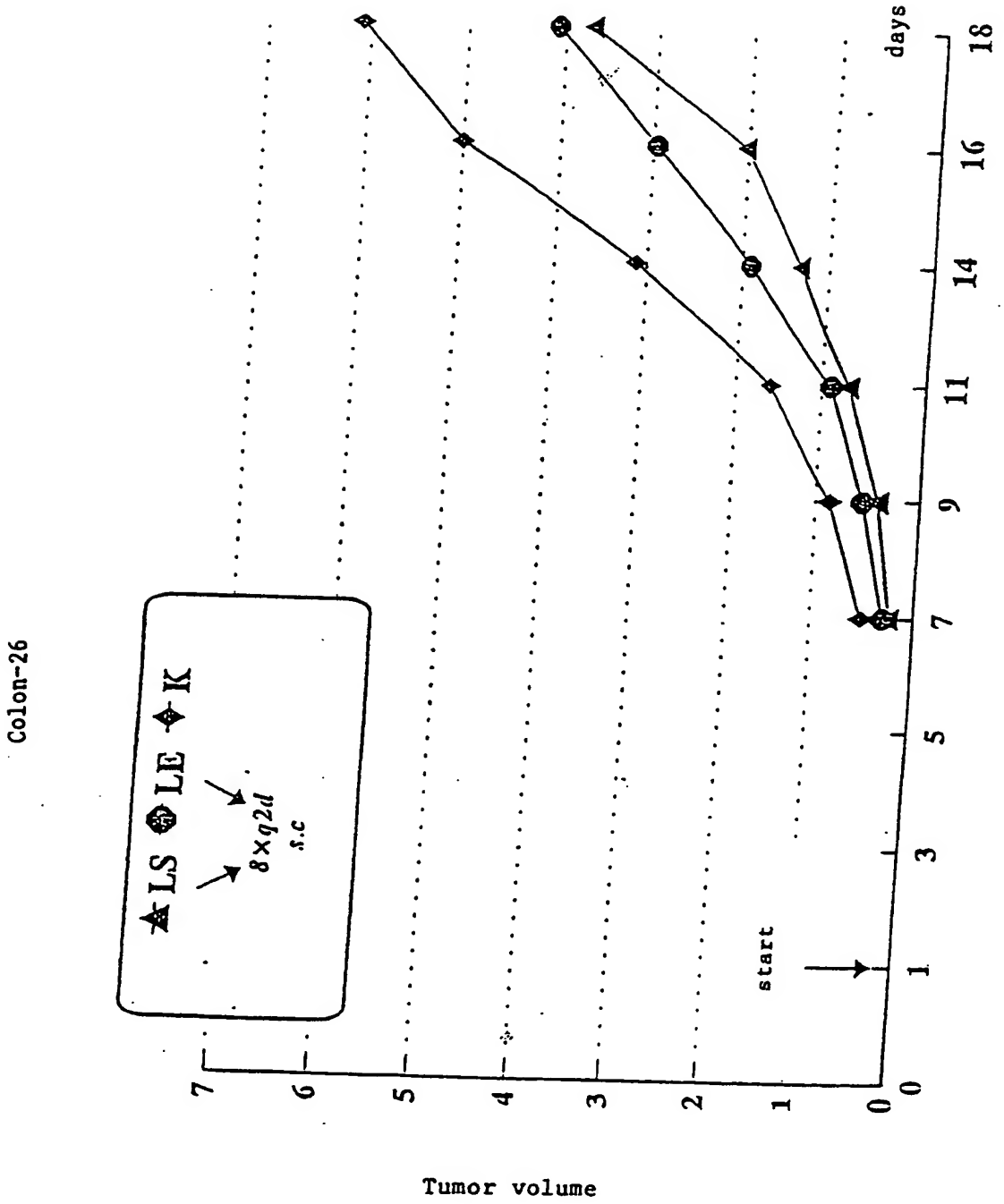

Fig. 7

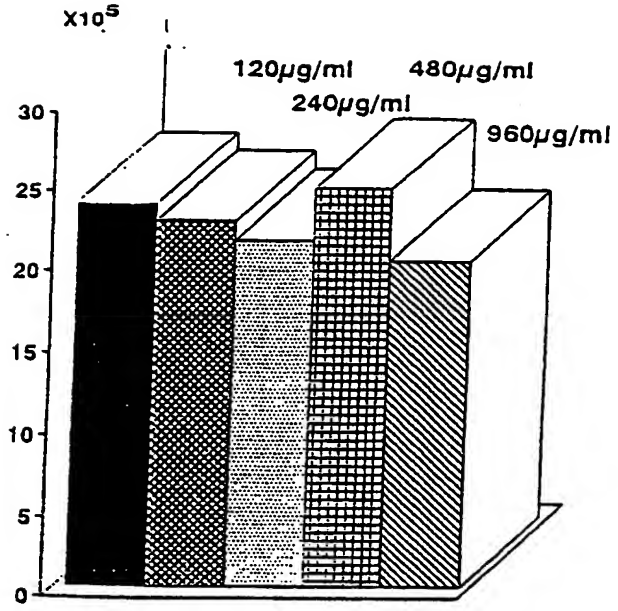

Fig. 8

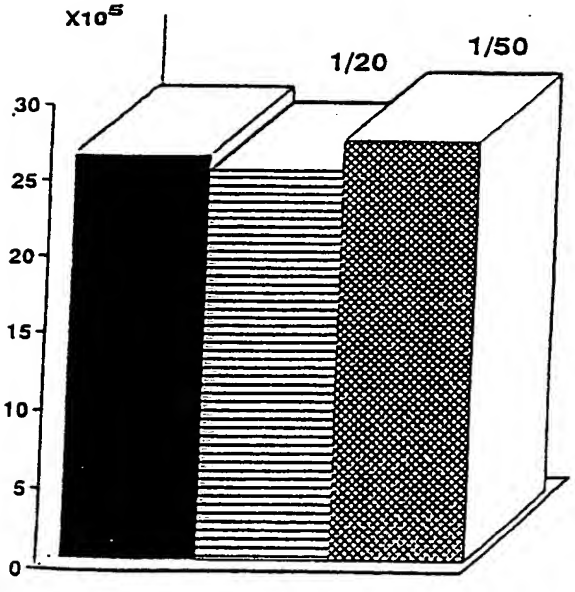

Fig. 9

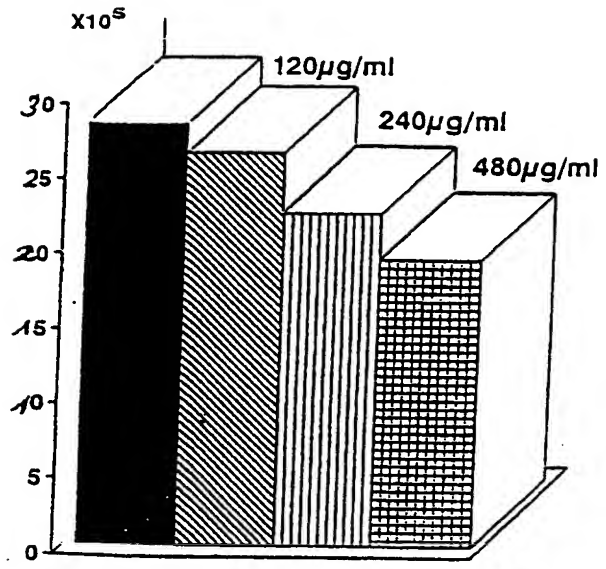

Fig. 12

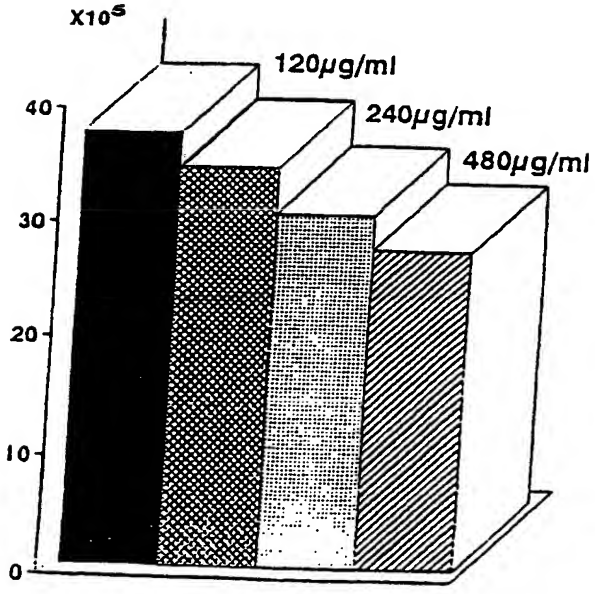

Fig. 13

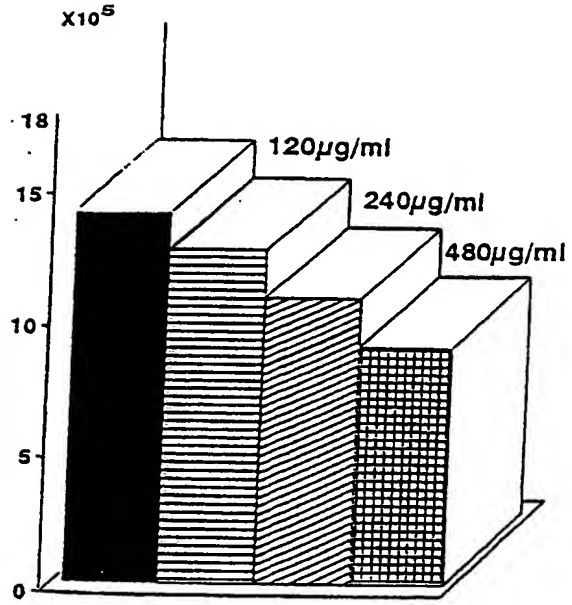

Fig. 16

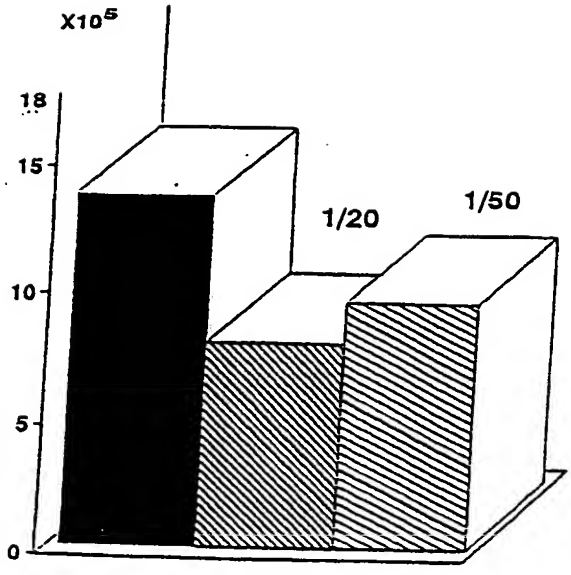

Fig. 17

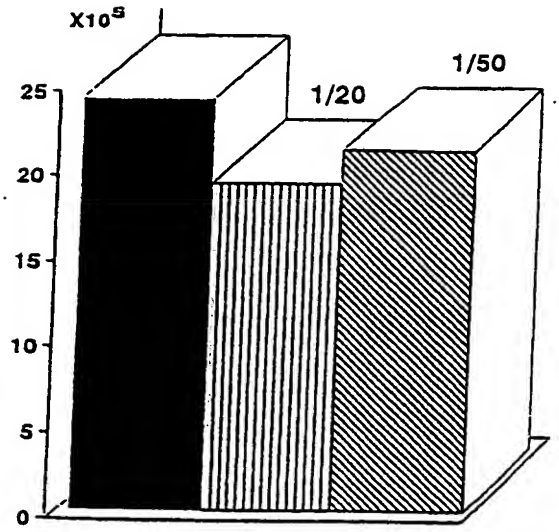

Fig. 20

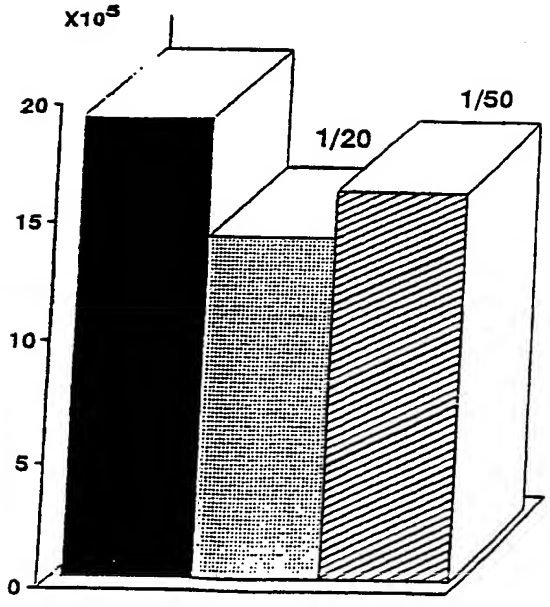

Fig. 21

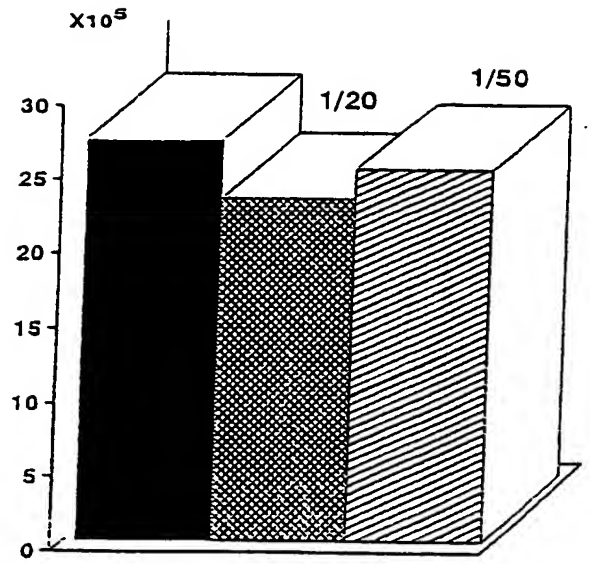

Fig. 24

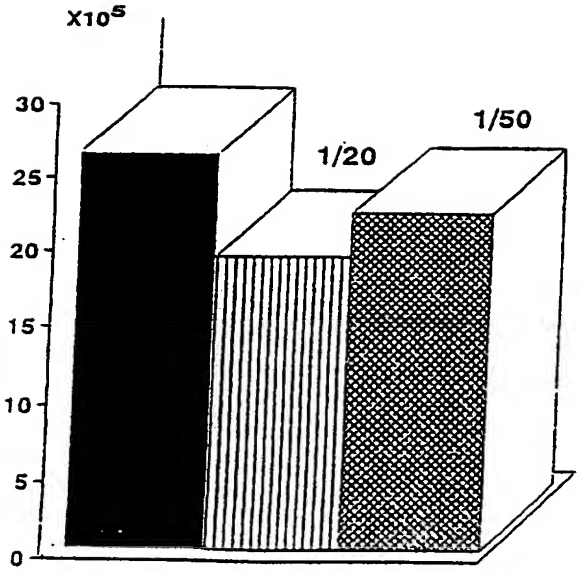

Fig. 25

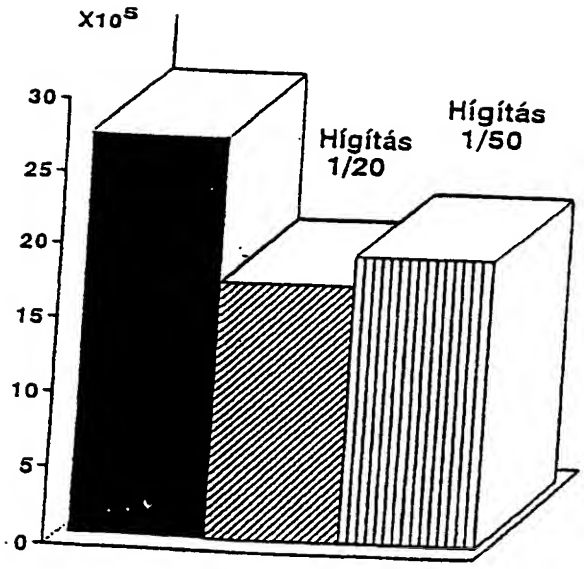

Fig. 28

The effect of normal serum (NS) on the growth of MCF-7 breast cancer cells

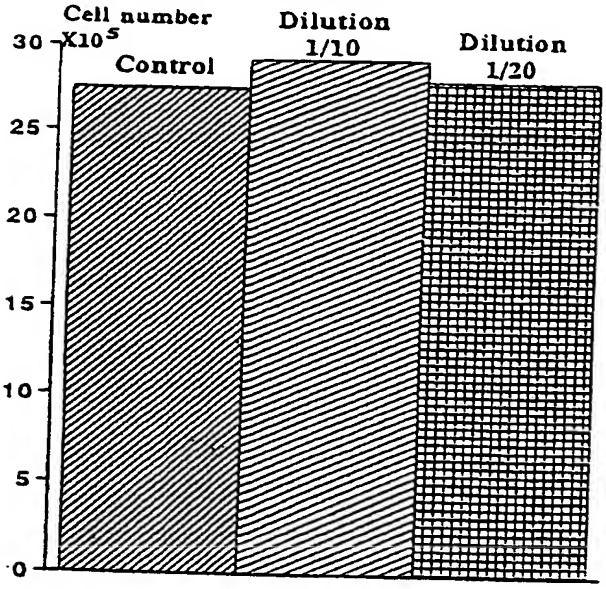

Fig. 31

The effect of leukemia serum (LS) on the growth of MCF-7 breast cancer cells

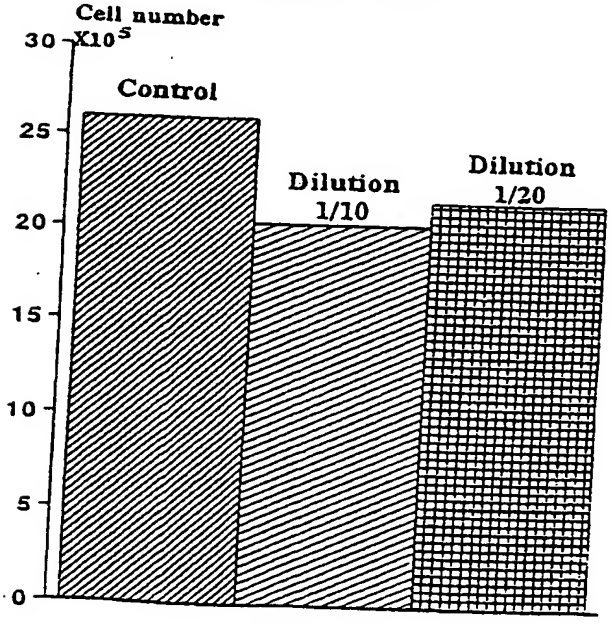

Fig. 32

The effect of the normal serum (NS) on the growth of C 26 colon carcinoma cells

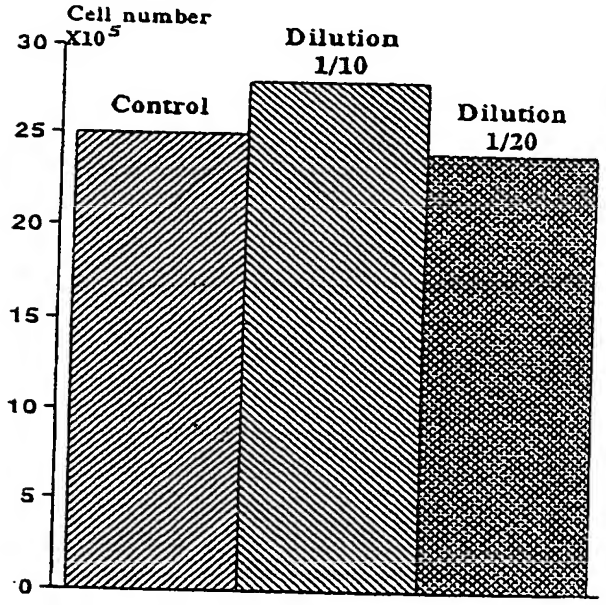

Fig. 35

The effect of the leukemia serum (LS) on the growth of C 26 colon carcinoma cells

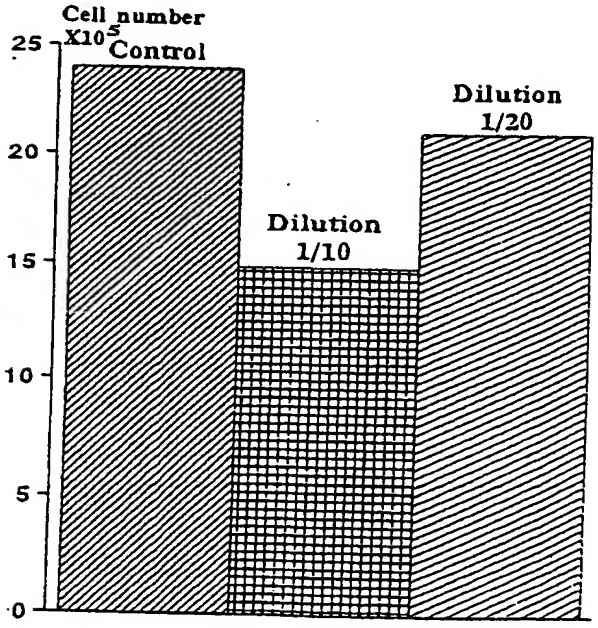

Fig. 36

The effect of normal serum (NS) on growth of the PC3 prostate cancer cells

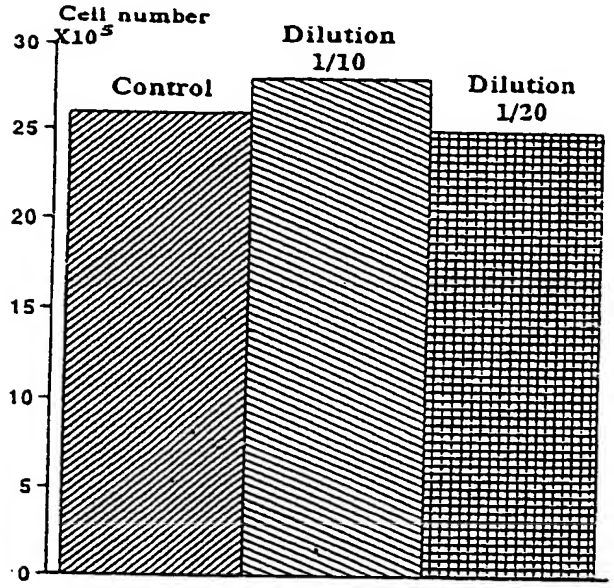

Fig. 39

The effect of the leukemia serum (LS) on the growth of PC3 prostate cancer cells

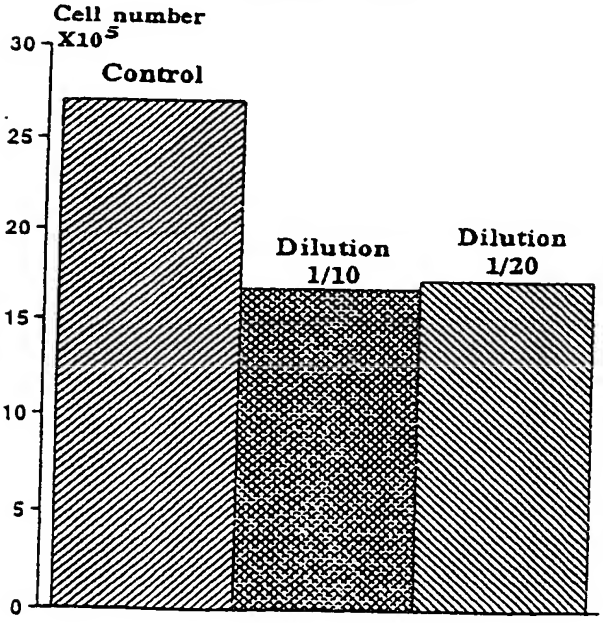

Fig. 40

The effect of the normal blood extraction (NE) on the growth of MCF-7 breast cancer cells

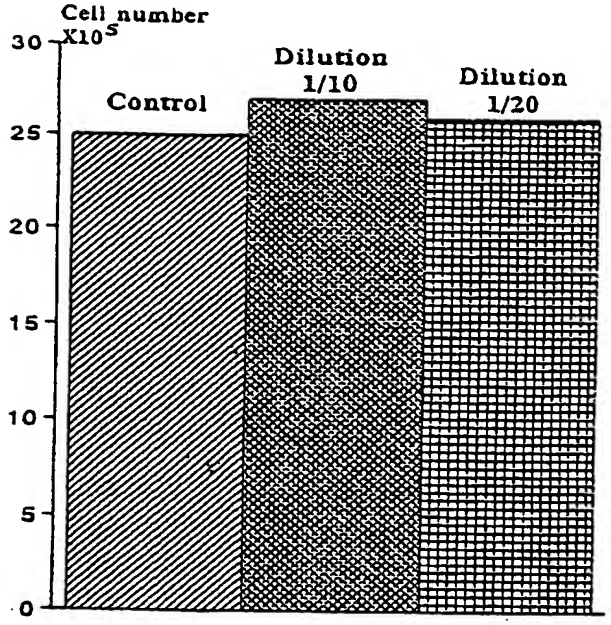

Fig. 43

The effect of the leukemia blood extraction (LE) on the growth of MCF-7 breast cancer cells

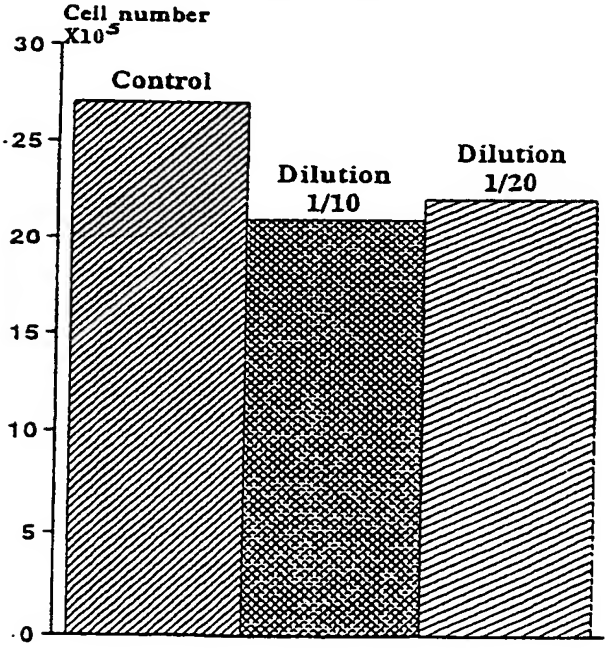

Fig. 44

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The effect of normal blood extraction (NE) on the growth of C 26 colon carcinoma cells

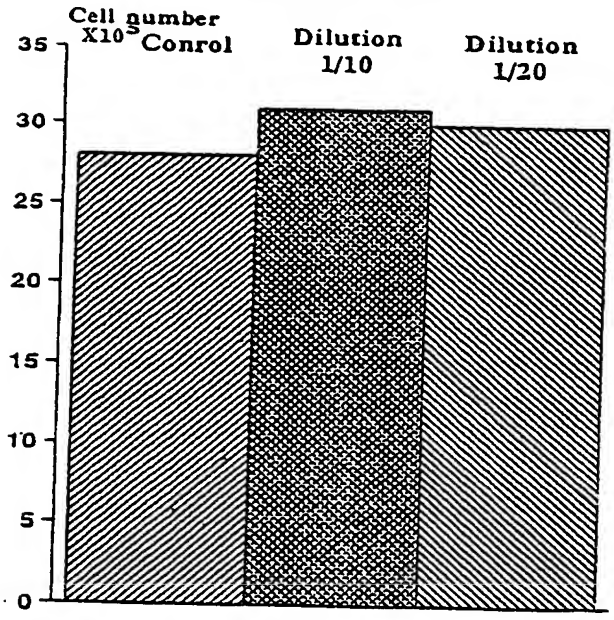


Fig. 47

The effect of leukemia blood extraction (LE) on the growth of C 26 colon carcinoma cells

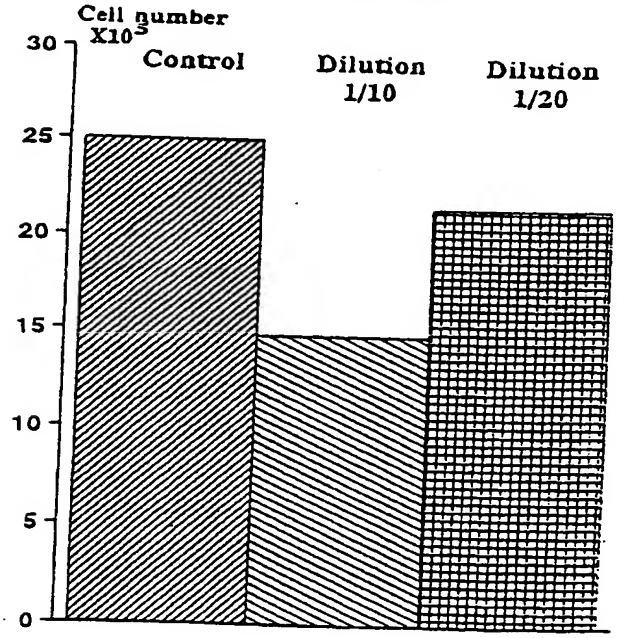


Fig. 48

The effect of the leukemia blood extraction (LE) on the growth of PC3 prostate cancer cells

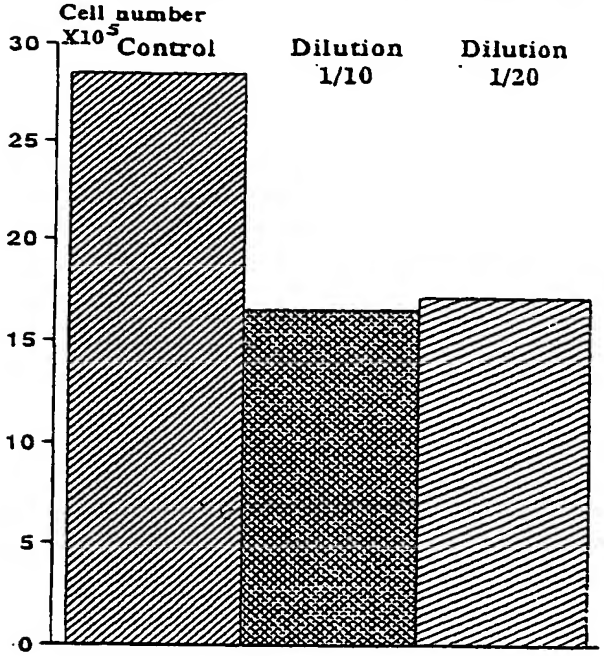


Fig. 51

The effect of the normal blood extraction (NE) on the growth of PC3 prostate cancer cells

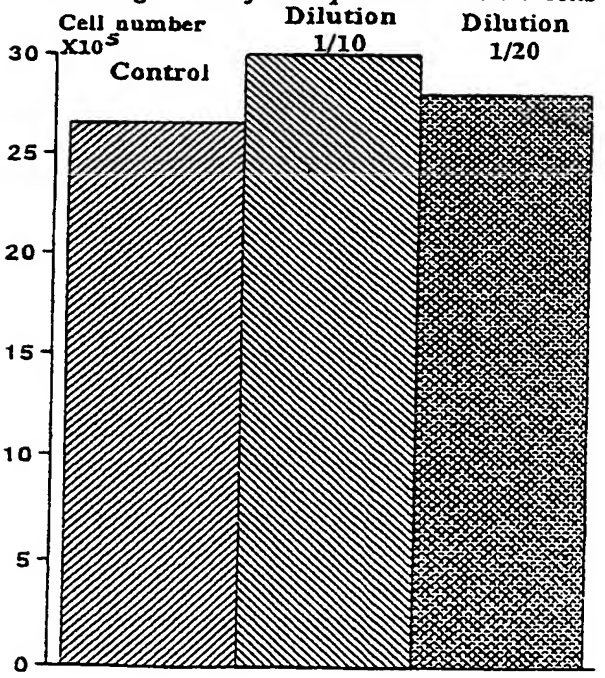


Fig. 52

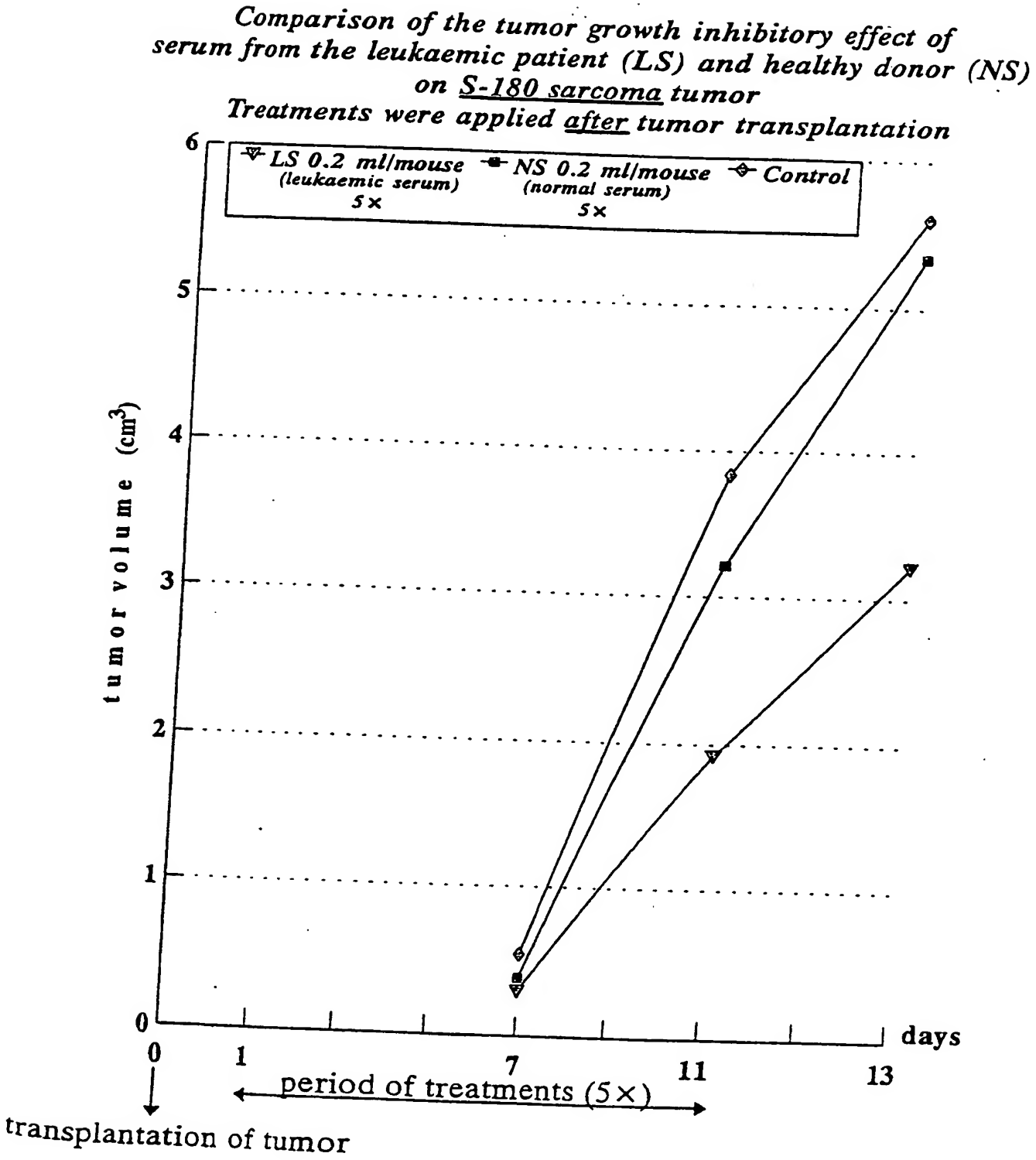


Fig. 54

Comparison of the tumor growth inhibitory effect of serum from the leukaemic patient (LS) and healthy donor (NS) on Colon-26 carcinoma

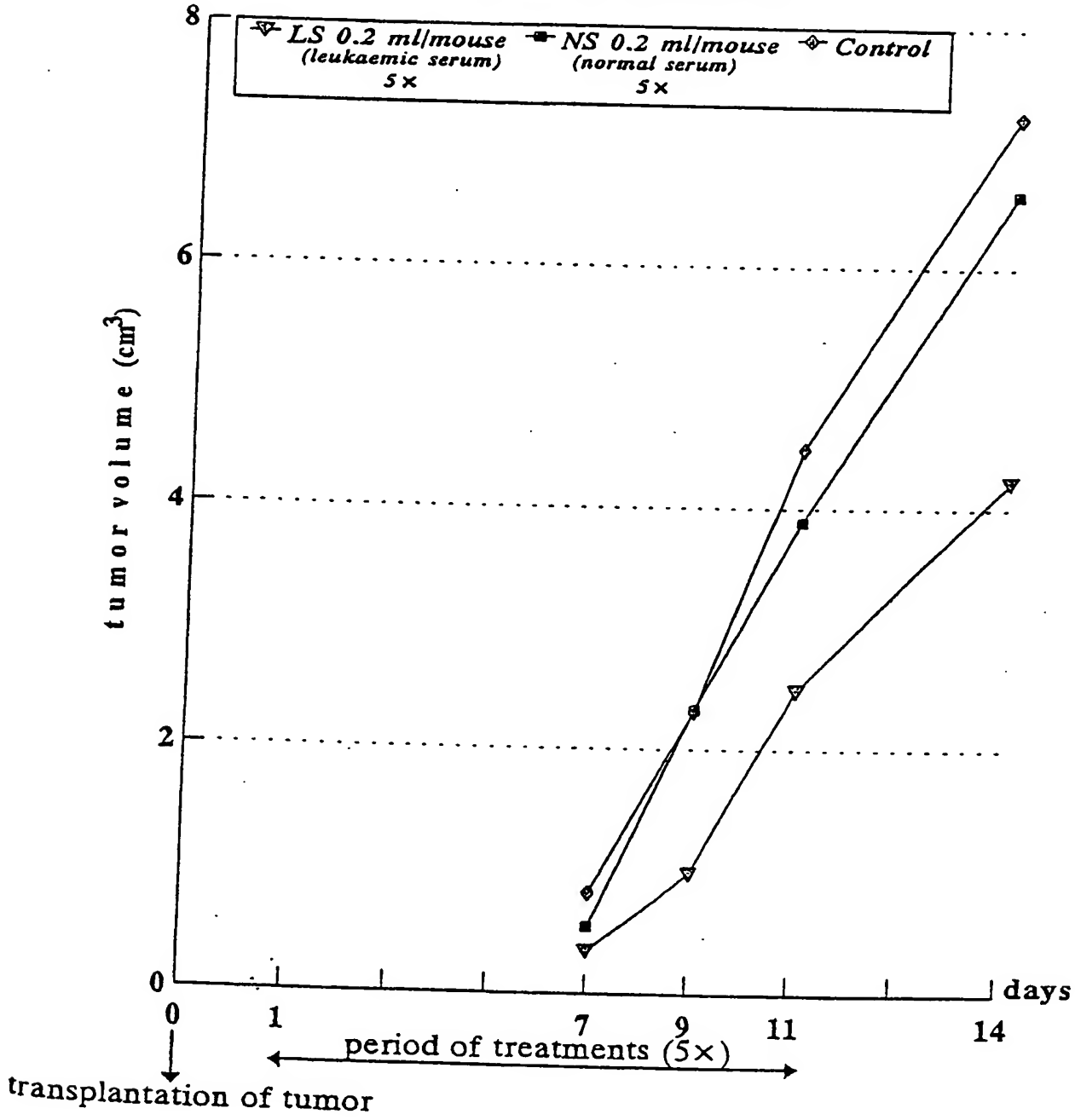
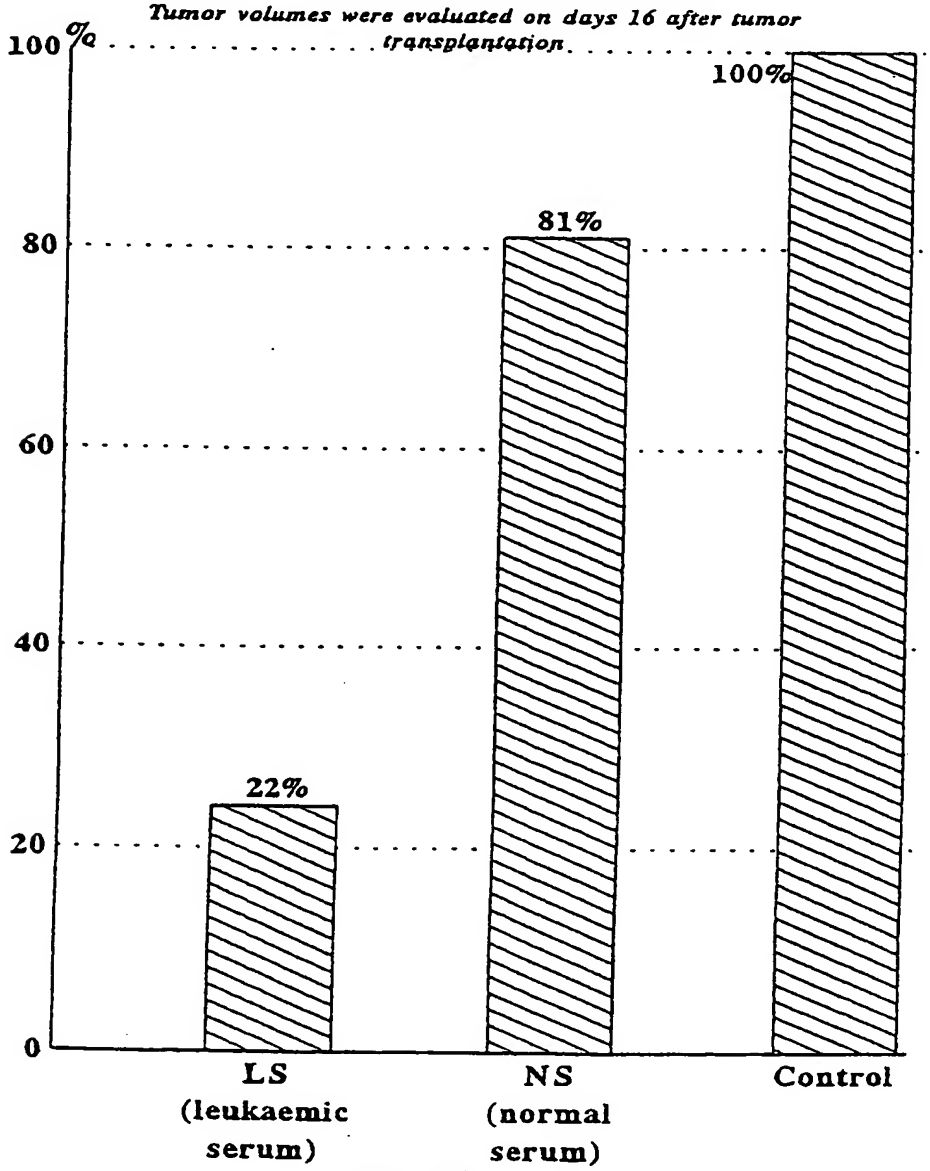


Fig. 56

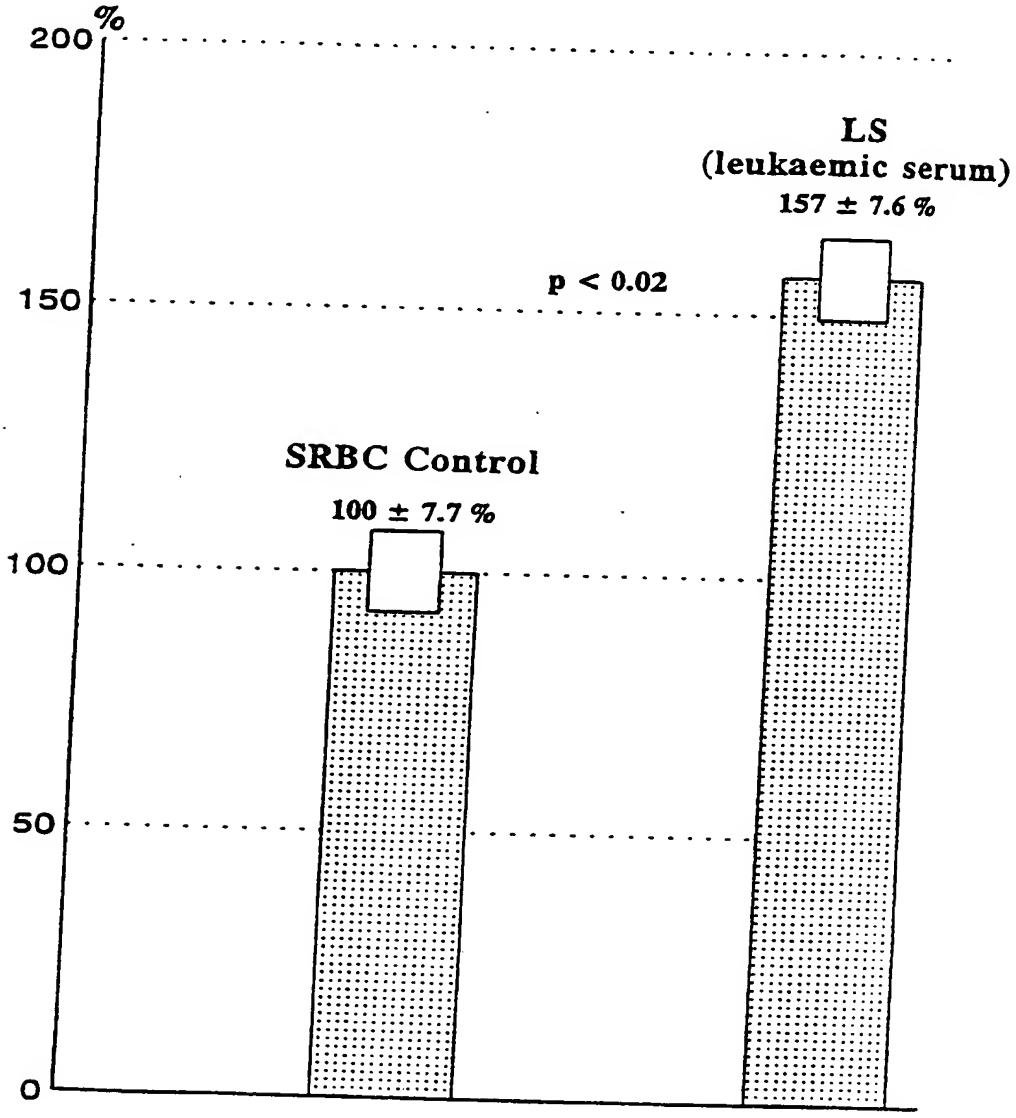
Comparison of the tumor growth inhibitory effect of serum from the leukaemic patient (LS) and healthy donor (NS) on P-388 s.c. mouse leukaemia



5×q2d
0.2 ml/mouse
Post-treatment

Fig.58

Influence of the leukaemic serum on the humoral immune response to SRBC antigen



SRBC Control = *group treated with sheep red blood cell antigen (1 × 10⁸ SRBC/mouse)*
LS = *group treated with leukaemic serum (0.2 ml/mouse) + treated with SRBC antigen simultaneously*

Fig. 60

Influence of the leukaemic serum (LS) on the humoral immune response of P-388 s.c. tumor bearing mice.
Immune response was evaluated on day 8 after tumor transplantation

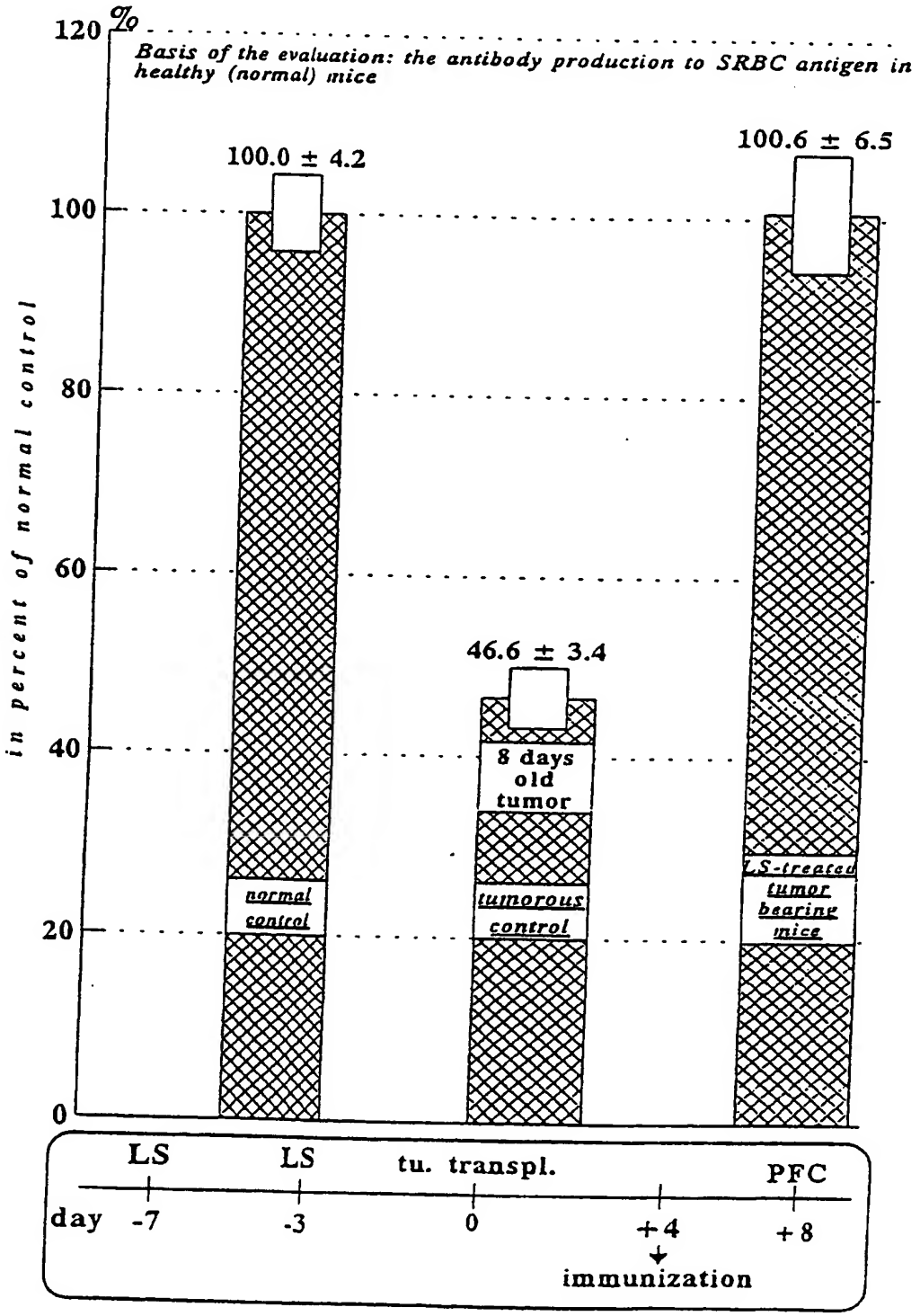


Fig. 62

Comparison of the tumor growth inhibitory effect of serum from the leukaemic patient (LS) and blood extract (LE) on S-180 sarcoma

Treatments were applied after tumor transplantation

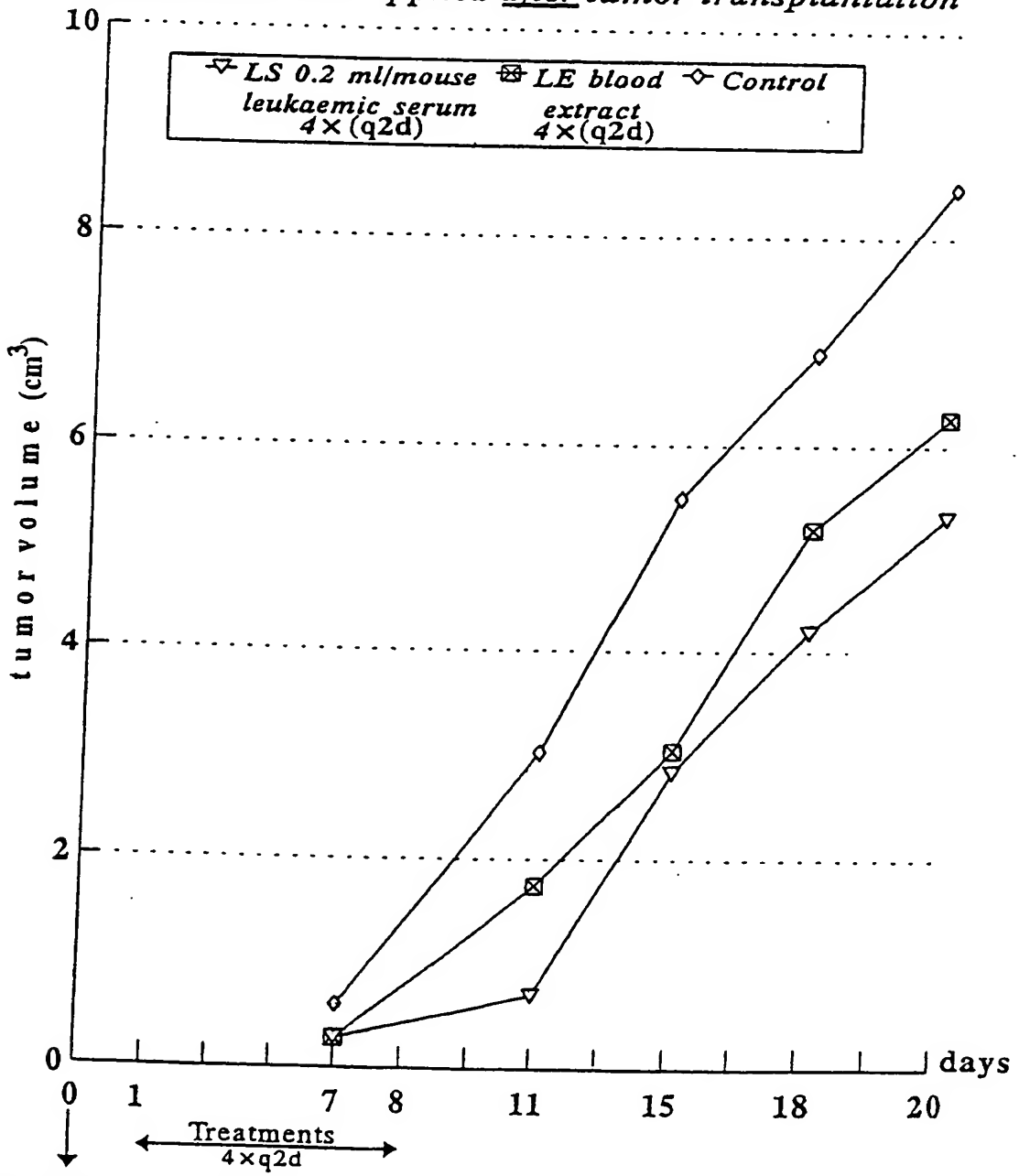


Fig. 64

Influence of leukaemic serum (LS) on the growth of P-388 s.c. leukaemia.
Treatments were applied before tumor transplantation

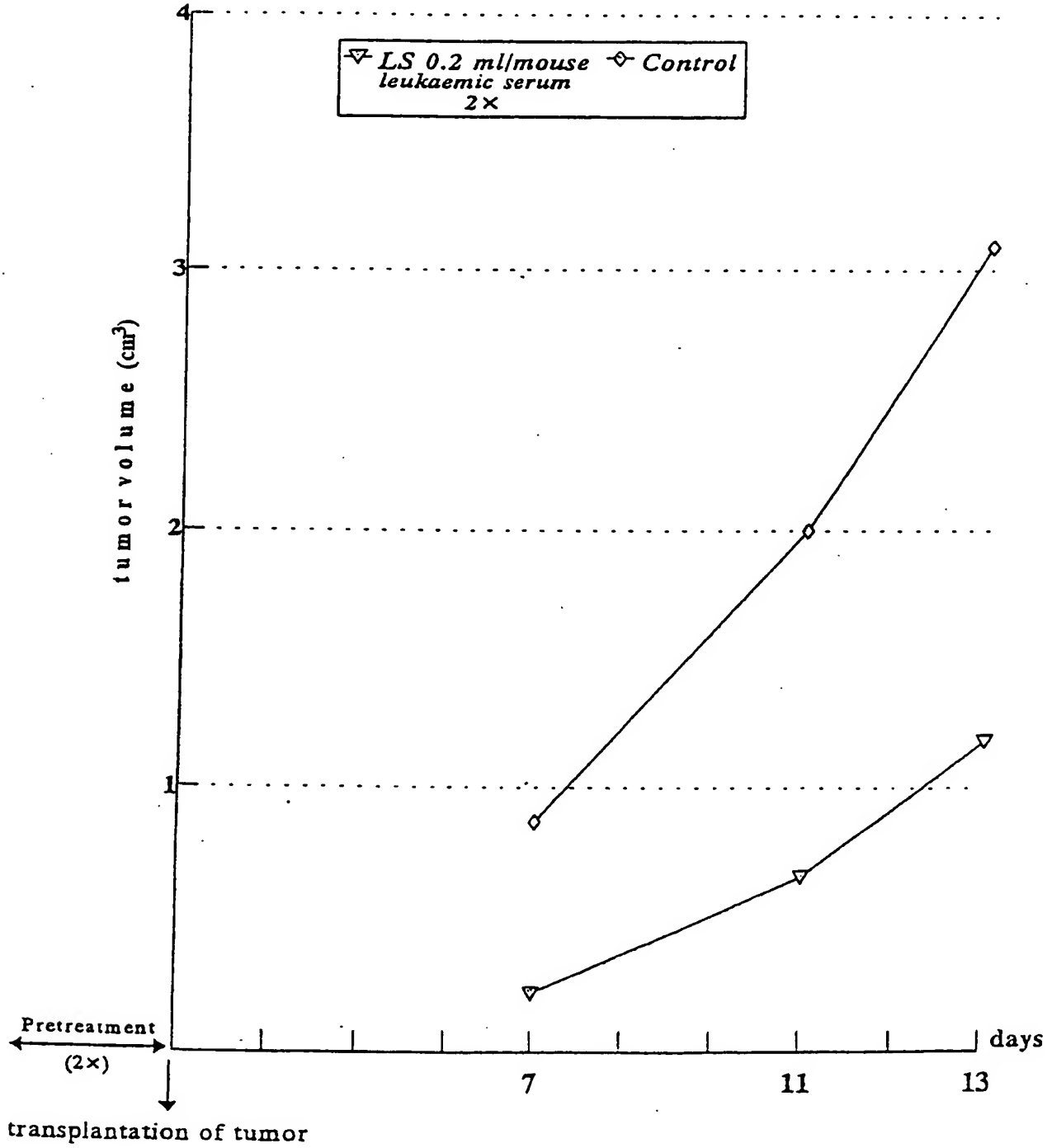


Fig. 66

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/HU 00/00002

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/14 A61P37/04 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MARTYN R: "ZUSAETZLICHE BEHANDLUNG DES KOLLUMKARZINOM IM III. STADIUM MIT INKUBIERTEN LEUKAEMIEBLUT NACH STRAHLENTHERAPIE" ZENTRALBLATT FUER GYNAEKOLOGIE,DE,JOHANN AMBROSIUS BARTH, LEIPZIG, vol. 93, no. 19, 1 January 1971 (1971-01-01), pages 634-639, XP002067385 ISSN: 0044-4197 cited in the application the whole document	1-10

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 June 2000	21/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Stein, A

Form PCT/ISA/210 (second sheet) (July 1992)